THE STRUCTURE AND INNERVATION OF THE SPHINCTERS IN THE LARGE INTESTINE OF THE DOMESTIC DUCK (Anas platyrhynchos)

by

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To my wife Ikbal and my daughters

Abeer, Areeg and Lamees

DECLARATION

I hereby declare that this thesis embodies the results of my own work, and that it has been composed by myself.

Adnan H. Mahdi

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I. INTRODUCTION

Birds form one of the main sources of animal protein for human consumption including meat and eggs. Whilst many factors influence this production, by far the most important of these is the efficiency of digestion in the intestinal tract. Digestion of food and the absorption of the products of this process are generally dependent on the rate at which food passes along the digestive tract, the secretory activity of the digestive glands being closely influenced by the rate of food passage. Movement of food is brought about by the gut muscle which is generally composed of an outer longitudinal layer capable of shortening the intestine and widening its lumen, and an inner circular layer which closes the lumen (DiDio and Anderson, 1968). Distributed along the digestive tract, in mammals at least, are thickenings of the circular muscle which on contraction constrict the gut, slowing down the flow of ingesta and preventing its retrograde movement (DiDio and Anderson, 1968; Reeve, 1981). These thickenings of the circular muscle are known as "sphincters". Delaying the passage of food along the digestive tract is essential to allow the various phases of physical and chemical digestion to occur. The basic aim of this thesis is to investigate the presence of anatomical sphincters in the digestive tract of birds and in particular the domestic duck (Anas platyrhynchos). Since a preliminary study of the literature has shown that in birds complex movements of the ingesta occur between the small and large intestines and between the caecum and rectum of the large intestine the study is restricted to the large intestine. As well

as providing information on the sphincters in the avian digestive tract it is hoped that the investigation will also contribute to an understanding of the morphology of gut sphincters in vertebrates in general, information which is vital for functional investigations of intestinal motility.

The anatomical nomeculature used in this thesis is based on the Nomina Anatomica Avium, 1979. The names of the common laboratory and domestic birds are as follows: duck or Anas, domestic forms of Anas platyrhynchos; goose or Anser, domestic forms of Anser anser; turkey or Meleagris, domestic forms of Meleagris gallopavo; chicken, domestic fowl or Gallus, domestic forms of Gallus gallus; quail, domestic forms of the genus Coturnix.

II. REVIEW OF LITERATURE

A. What is a Sphincter?

The term " sphincter " originates from the Greek word sphingein meaning " to bind tight ". Three criteria have been used to describe sphincters. These are: (i) their anatomy; (ii) their physiological function; and (iii) their pharmacological response.

The term sphincter is defined anatomically as a thickening of the circular muscle layer which controls the opening of a body orifice or constricts the lumen of a natural body passage (Lendrum, 1937; Botha, 1958 c). In purely physiological terms a sphincter is an area of muscle in the gastrointestinal tract which tonically closes and has the ability to relax and contract (Thomas and Mann, 1981 p. 227). Sphincters can also be defined pharmacologically as a muscular area where sympathetic activity produces contraction and parasympathetic activity induces relaxation (Thomas and Mann, 1981 p. 227). However, it should be noted that not all of these criteria can be applied to the sphincters in the gastrointestinal tract of mammals, the vertebrate group in which the sphincters have been most frequently studied, since these definitions are used for smooth muscle sphincters and not to those containing striated muscle. In this thesis the term "sphincter" is used for a structure formed by smooth muscle only.

One feature of the smooth muscle sphincters in the mammalian digestive

tract is that not all the criteria used for sphincters appear to be valid for each one. In addition, the dominant features exhibited by each sphincter may differ markedly from one sphincter to another. For example, although in man a sphincter cannot be anatomically identified at the gastro-oesophageal junction (Higgs et al., 1965), manometric and radiologic studies have shown the existence of a sphincter at this site (Botha et al., 1957; Code et al., 1958) which has different pharmacological responses to that of the smooth muscle in the adjacent oesophagus (Bass et al ., 1970). In contrast, the markedly thickened circular pyloric muscle at the gastro-duodenal junction in man has the anatomical appearance of a sphincter (Torgersen, 1942; Loukes et al., 1960), although its motility is not different from that of the antral muscle (Atkinson et al., 1957) and its pharmacological responses are the same as that of the adjacent musculature (Bass et al., 1970).

Whilst many investigators consider that muscle is the major tissue responsible for sphincter activity, Stieve (1928, 1930) has shown that although muscle provides the contractibility of a sphincter it is not the only tissue involved in changing the size of the lumen of the alimentary tract. The density of the nerve supply of the muscle (Vaithilingam et al., 1984), the amount of collagenous connective tissue (Horton, 1928) and elastic tissue (Nagel, 1938), and the presence of mucosal folds (Botha, 1958 a) may all play a role.

B. Methods Used to Study the Anatomy of the Digestive Tract Sphincters.

Nearly all the information on the anatomical techniques which have been used to study gut sphincters is present in the literature on mammals since this is the only vertebrate class in which the digestive tract sphincters have been seriously investigated. Four anatomical approaches have been used to varying extents. These include gross observations, routine histological techniques, the reconstruction of photographic 3-dimensional models, and morphometry.

(1) Gross observations.

This is the earliest and most traditional method used to study the existence of muscular thickenings at the junction regions in the intestinal tract and involves observations on fresh or fixed specimens with or without the aid of a dissecting microscope. The approach was used in man, for example, by Horton (1928) and Torgersen (1942) to demonstrate the massive thickening of the musculature at the pylorus.

(2) Routine light microscopy.

By means of routine histological techniques the existence or otherwise of localised thickenings of the circular muscle layer has been demonstrated. The approach was used by Botha (1958 b) in the rabbit and bat and by Vaithilingam et al. (1984) in the monkey to demonstrate a sphincter at the gastro-oesophageal junction. Conversely, the technique was not able identify a sphincter at the same site in man (Lendrum, 1937)

(3) Photographic reconstruction models.

This method is relatively recent and was pioneered by Los (1970) and Langemeijer and Simon (1973). The preparation of these models to demonstrate the existence of gut sphincters has been used by Jackson (1978) to show the presence of a spiral constrictor at the gastro-oesophageal junction in human infants and by Vaithilingam et al. (1984) to demonstrate that there is a thickening of the muscle at the gastro-oesophageal junction in the monkey. The method involves serial photography of every transverse microscopic section. The negatives are then enlarged and printed. All parts of the photograph which are not needed in the reconstruction are excised. In order that the length magnification of the preparation is the same magnification as the width which has been increased during the enlarging and printing of the negatives, the original thickness of

the microscopic sections is multiplied by the photographic enlarging factor. To reach the appropriate length of the preparation cardboard of suitable thickness is fixed to the back of each photograph.

The trimmed photographs are piled on top of each other in series and their position in the model is adjusted by the use of reference points (Langemeijer and Simon, 1973) which are introduced by inserting steel pins into the embedding mould close to the specimens.

(4) Morphometry.

This approach is based on transmission electron microscopy and was utilised by Vaithilingam et al.(1984) to demonstrate the existence of sphincters at the gastro-oesophageal and gastro-duodenal junctions in the monkey. It involves estimating the density of the innervation of the muscle as revealed by the number of nerve bundles and vesiculated axon profiles per number of circular muscle cells and comparing the innervation of the muscle in the region of the gut under investigation with that of the muscle from an adjacent area.

C. Evidence for Sphincters in the Large Intestine of Birds.

Compared with mammals the evidence for sphincters in the avian digestive tract is extremely limited. In the large intestine investigations have centred

on the ileo-caeco-rectal junction and the recto-coprodeal junction but as shown below the observations on these regions are conflicting.

(1) Ileo-caeco-rectal junction.

In the majority of avian species the right and left caeca arise from the rectum close to the junction with the ileum and usually in the lateral wall of the intestine. The extent to which the two caeca are developed is characteristic of each major group of birds (McLelland, 1979). Galliform species have relatively long caeca (McLelland, 1979) and all the investigations have been restricted to birds in this order. In the chicken a distinct ridge can be seen on the external surface of the gut at the junction between the ileum and the rectum (Clarke, 1978) and the mucosa of the terminal part of the ileum projects slightly into the rectal lumen (Hodges, 1974, p. 81; Clarke, 1978). The entrance of each caecum is narrow and contains folds of mucous membrane with prominent villi (Browne, 1922; Clarke, 1978). In general, the muscle layers in the ileum, caecum, and rectum are basically similar and consist of a muscularis mucosae, an inner circular layer, and an outer longitudinal layer (Calhoun, 1954). The circular muscle layer of the chicken ileum consists of an inner thin portion and an outer thick portion (Clara, 1926; Gabella, 1985). The muscle cells of the inner portion are smaller and less electron dense than those of the outer portion. According to Calhoun (1954) the circular muscle of the ileum in the domestic

fowl is thickened at the ileo-rectal junction and at the origin of each caecum, and Clarke (1978) observed a well-developed muscular ring within the ileal projection. These appear to be the first observations on anatomical sphincters in this region of the avian gut although the evidence presented by the authors is slight and lacking in detail. At the level of the caecal openings according to Clarke (1978) the musculature is complex and consists of parts of the circular muscle of the ileum, rectum and caeca. He was not, however, able to describe in any detail the arrangement of the muscle at the origins of the caeca and its relationship to the muscular ring at the ileo-rectal junction. He concluded that the terms " valve " or "sphincter " should be avoided at this region because of their functional implications. Hodges (1974, p. 81) found that immediately cranial to the ileo-rectal junction in the chicken the muscularis mucosae is also thickened.

A slight thickening of the circular muscle at the origin of the caeca in the Japanese Quail (Coturnix japonica) was observed by Fenna and Boag (1974 a). In the quail according to Fenna and Boag the caecal wall projects for a short distance into the rectal lumen. In contrast, Fenna and Boag (1974 b)were not able to observe any thickening of the circular muscle at the origins of the caeca in the Spruce Grouse (Canachites canadensis).

There is no clear physiological evidence for the existence of sphincters at the ileo-caeco-rectal junction in birds. In mammals conclusive evidence for the

existence of physiological sphincters in the gut have been obtained from measurements of intraluminal pressure (Atkinson et al., 1957) which showed zones of high pressure at the gastro-oesophageal (Fyke et al., 1956; Atkinson et al., 1957) and pyloric junctions (Fisher and Cohn, 1973). Such precise and unequivocal data have not been obtained for birds. Instead nearly all the physiological work has been on the general function of the avian hindgut.

From radiographic evidence, it is generally accepted that in the chicken retroperistaltic waves originating in the cloaca move the intestinal contents cranially into the rectum and caeca but do not enter the ileum. On the basis of these observations a sphincter-like activity has been postulated in the region of the ileo-caeco-rectal junction although precise details of the mechanism are not available (Yasukawa, 1959; Akester et al., 1967; Nechay et al., 1968; Fenna and Boag, 1974 a; McLelland, 1979 , p. 149; Hill, 1983, p. 32). Furthermore, it is suggested that both ends of the rectum may be closed allowing the rectal contents to pass into the caeca through the caecal orifices by continuous retroperistaltic rectal contractions (Hill, 1983, p. 32). However, Tindall (1976) was not able to demonstrate any co-ordination between the activities in the caudal end of the rectum at the recto-coprodeal junction and in the cranial end of the rectum at the ileo-rectal junction.

Overall the evidence, both anatomical and physiological, for the presence of sphincters at the ileo-caeco-rectal junction in birds is not convincing and

requires further study. No anatomical or physiological observations appear to have been made on the existence of sphincters at the ileo-caeco-rectal junction in the domestic duck.

(2) Recto-coprodeal junction. *

The caudal part of the rectum in birds opens into the cranial compartment of the cloaca, the coprodeum. Usually the only evidence which is put forward for the existence of a barrier between the rectum and cloaca is the presence of a mucosal fold between the two parts of the gut. However, there is considerable debate in the literature as to whether or not such a fold exists and it appears that there are many species differences. Thus, a well-developed fold has been described at the recto-coprodeal junction in the Ostrich (Struthio camelus) (Saint-Hilaire, 1822; Gadow, 1887; Jolly, 1915), although it was not seen in other ratites including the Emu (Dromaius novaehollandiae) (Saint-Hilaire, 1822) and Casuarius and Rhea (Retterer, 1885; Gadow , 1887). In the domestic fowl there is much discussion as to whether or not a true macroscopic fold is present. Thus whilst it has been described by many workers including Pilz (1937), Grau (1943), Lucas and Stettenheim (1965), Akester et al.(1967), Hill (1971, p. 20) and Preuss and Rautenfeld (1974) other authors have not been able to find it (Lereboullet, 1851; Jolly, 1915; Lillie, 1952, p. 388; Komarek; 1970; King, 1975, p. 1961). Amongst anseriforms a fold occurs at

* most of the information in this section has been cited by King (1981, p. 72).

the junction in some species but not in others, and some of the evidence suggests that intraspecific variations exist. Thus Liebe (1914) found that a fold was prominent in the domestic duck (Anas platyrhynchos), and a low but conspicuous circular ridge was also observed in the male domestic duck and goose by Komarek (1969). In contrast, King (1981, p. 72) did not observe a true macroscopic fold at the recto-coprodeal junction in the males and females of the domestic forms of Anas, Anser, Cairina moschata, in the male Mute Swan (Cygnus olor) and Pinkfooted Goose (Anser fabalis), or in the female Mallard (Anas platyrhynchos). Nevertheless he observed a conspicuous change in the gross appearance of the mucosa at the junction, the coprodeal mucosa being slightly raised.

Histological evidence in the literature for a muscle boundary between the rectum and coprodeum does not appear to be available in any species.

On the basis of their radiographic study Akester et al. (1967) concluded that in the chicken the junction between the coprodeum and rectum is closed during periods when the coprodeum is quiescent. Weyrauch and Roland (1958) also found that closure of the junction occurs and is completely effective in preventing reflux from the cloaca into the rectum against pressure of up to 40 mm of H2O.

D. Anatomical Evidence for Sphincters in the Digestive Tract of Mammals.

Since there is minimal information in the literature on the distribution and structure of gut sphincters in birds the anatomical evidence for sphincters in the digestive tract of mammals will be analysed with a view to ascertaining their position in the gut and the arrangement of their components. These data it is hoped will provide a background of information which can be drawn upon in the present study. It is generally agreed that there are seven smooth muscle sphincters in the digestive tract of mammals. These are the palatopharyngeal sphincter, the cricopharyngeal sphincter,the gastro-oesophageal(cardiac) sphincter, the pyloric sphincter, the sphincter of Oddi, the ileo-caecal valve, and the internal anal sphincter. The present review is restricted to three of the best documented of these: the gastro-oesophageal sphincter, the pyloric sphincter, and the ileo-caecal valve.

(1) Gastro-oesophageal sphincter.

This sphincter is found at the junction formed by the abdominal part of the oesophagus and the cardiac part of the stomach (DiDio, 1948, 1949). Many approaches have been used to study this junction in both man and lower mammals including (a) gross observations, (b) light microscopy, (c) the preparation

of reconstruction models, and (d) morphometry.

(a) Gross observations.

A change in the colour of the mucosa is believed by many investigators to indicate the position of the gastro-oesophageal junction. In man the mucosa at the junction changes from a smooth, white and opaque surface in the oesophagus to a pink surface in the stomach (Lendrum, 1937). According to Botha (1958 a) well-developed mucosal folds are found at the gastro-oesophageal junction in man, rabbit, bat, and tortoise. In the other animals he investigated, consistent and regular folds were not present at the cardia. Jackson (1978) dissected the junction in human infants and adults and found no gross thickening of the circular muscle. However, he demonstrated that the circular muscle layer of the oesophagus gives rise to the oblique muscle layer of the stomach, the muscle fibres crossing the junction obliquely. He concluded that in man the gross evidence suggests that whilst a circular sphincter is not present, there is a sphincter-like spiral constrictor formed by the oblique fibres.

In his comparative anatomical study of the gastro-oesophageal junction Botha (1958 c) showed that whilst in some species i.e. cat, dog, sheep and ox there is no macroscopic thickening of the muscle layer at the junction, in others a powerful anatomical sphincter is present which can be clearly demonstrated grossly as either a localized thickening of the circular muscle as in the

bat and rabbit or a diffuse increase in the thickness of the muscle as in the pig and horse. According to Dyce et al. (1987, p. 511) the gastro-oesophageal sphincter in the horse is exceptionally well-developed. In the animals in which the macroscopic thickening of the muscle is not apparent, the caudal end of the oesophagus is narrow and at the cardia of the stomach is somewhat constricted (Botha, 1958 c).

(b) Light microscopy.

The oesophagus is a muscular tube with species differences in the type and distribution of the muscle. In man, primates, and marsupials the proximal one-third of the oesophagus is striated and the remainder is smooth muscle. In the dog, in contrast, the entire musculature of the oesophagus is striated (Thomas, 1981, p. 76).

Histologically in man the oesophageal musculature in the region of the gastro-oesophageal junction shows no evidence of localized thickening (Lendrum, 1937; Jackson, 1978). In addition Lendrum (1937) was not able to demonstrate any thickening of the elastic tissue at the lower end of the oesophagus which could influence sphincter function at this junction.

In animals there appears to be considerable interspecific histological variation in the structure of the gastro-oesophageal junction. Whilst a thick muscular

layer was described at the junction in the rat, hamster, bat, rabbit, pig, and horse by Botha (1958 c) and in the monkey by Vaithilingam et al. (1984), such a thickening of the muscle layer was not observed by Botha (1958 c) in the cat, dog, sheep and ox. According to Mann and Shorter (1964) the caudal third of the oesophagus in the dog has a substantial muscularis mucosae of smooth muscle which is present in the mucosal folds at the gastro-oesophageal junction. Furthermore, the inner circular layer of striated muscle in the last 1-2 cm of the oesophagus is replaced abruptly by a thick circular layer of smooth muscle and the outer longitudinal layer of striated muscle continues below this point for a distance of 2-3 cm. The thickening of the circular smooth muscle and the existence of mucosal folds containing a well-developed muscularis mucosae at the caudal end of the oesophagus in the dog form a distinct anatomical basis for a gastro-oesophageal sphincter. The precise level at which the muscle is thickened at the junction varies. Thus it is thickened slightly caudal to the cardia in the pig, at the level of the cardia in the bat, and cranial to the cardia in the rat and hamster (Botha, 1958 c).

(c) Reconstruction models.

Reconstruction models of the musculature at the gastro-oesophageal junction have been made for man by Jackson (1978) and for the monkey, Macaca fascicularis, by Vaithilingam et al. (1984). In man the model confirmed the

gross observations that the inner circular muscle layer of the oesophagus gives rise to a bundle of muscle fibres which descends obliquely across the junction and becomes the oblique muscle layer of the stomach. In the model prepared by Vaithilingam et al. (1984) of the junction in the monkey, Macaca fascicularis, a thickening of the circular muscle was clearly observed.

(d) Morphometry.

The only morphometric study on the innervation of the circular muscle of the gastro-oesophageal junction appears to be that by Vaithilingam et al. (1984) in the monkey, Macaca fascicularis. This investigation showed that the number of nerve bundles and the percentage of the vesiculated axon profiles was statistically greater in the circular muscle of the junction than in the muscle in the body of stomach.

(2) Pyloric sphincter.

This sphincter is found at the junction between the antral part of the stomach and the proximal part of the duodenum (Horton, 1928; Torgersen, 1942; DiDio and Anderson, 1968, p. 95; Johnson, 1981, p. 101; Cai and Gabella, 1984). Anatomically the sphincter has been studied by gross observations, light microscopy and morphometry.

(a) Gross observations.

The macroscopic existence of a sphincter at the gastro-duodenal junction has been investigated in man, ox, horse, pig and guinea-pig.

In man the junction between the stomach and the duodenum is marked grossly by a thick ring of circular muscle just aboral to the point where the epithelium of the stomach becomes the epithelium of the duodenum (Horton, 1928; Johnson, 1981, p. 101). The muscular ring is not uniformly developed but gradually thickens from its proximal end to its distal end. The gastric mucosa is very mobile in the antrum of the stomach and projects into the duodenum to a varying extent rather like the projection of the uterine cervix into the vagina. The part of the projection arising on the side of the greater curvature extends more into the duodenum than the part arising on the side of the lesser curvature (Horton, 1928; Johnson, 1981, p. 104). Horton (1928) showed that in the foetus and newborn infant the mucosal projection contains part of the thickened sphincter muscle.

In the horse the circular muscle is also greatly thickened at the gastro-duodenal junction forming a muscular ring (Sisson, 1975, p. 479). The position of this ring is indicated externally by a distinct constriction and internally by a circular ridge caused by the thickened muscle tissue. In the ox a thick protru-

sion of the circular muscle projects from the distal end of the lesser curvature of the antrum into the pyloric cavity at the junction with the duodenum. This projection is known as the "torus pyloricus " (Habel, 1975, p. 898). In the pig a thick muscular protrusion was also described at the pylorus by Sloss (1954) and appears to be formed from the circular and oblique muscle layers of the stomach. Great thickening of the circular muscle in the terminal part of the stomach in the guinea-pig was observed grossly by Cai and Gabella (1984), the transition between the stomach and duodenum being marked externally by a shallow circular groove.

(b) Light microscopy.

Histological studies of the gastro-duodenal junction have provided evidence for the presence of a thick muscular ring in man (Horton, 1928; Torgersen, 1942) and in the rabbit, cat, dog, ox and horse (Torgersen, 1942).

In man (Horton, 1928) and the guinea-pig (Cai and Gabella, 1984) the thickened pyloric sphincter muscle is formed only by the circular muscle of the stomach since there is a connective tissue septum separating this thickened gastric muscle from the circular muscle of the duodenum. In the animals investigated by Torgersen (1942), the separation between the musculature of the sphincter and the duodenal circular muscle is found only at the greater curvature, whilst at the lesser curvature the gastric muscle is continuous with that of the duode-

num. In all the mammals investigated the longitudinal muscle of the antrum fuses with the thickened pyloric circular muscle except at the lesser curvature where some of the longitudinal muscle fibres of the antrum cross the junction and merge with the longitudinal muscle of the duodenum (Horton, 1928; Torgersen, 1942; Cai and Gabella, 1984).

(c) Morphometry.

The density of innervation of the muscle at the gastro-duodenal junction was studied by Cai and Gabella (1984) in the guinea-pig and Vaithilingam et al. (1984) in the monkey, Macaca fascicularis. These investigations showed that the density of innervation including the number of nerve bundles and the percentage of varicosities per number of muscle cell profiles was higher in the pyloric part of the stomach than in the duodenum and antrum in the case of the guinea-pig and in the body of the stomach in the case of the monkey.

(3) Ileo-caecal valve.

This sphincter is found at the site where the terminal part of the ileum opens into the large intestine (DiDio, 1952, Reeve, 1981 p. 14). The anatomy of this junction has been investigated by gross observations and light microscopy.

(a) Gross observations.

Most of the gross anatomical studies of the ileo-caecal junction have shown that the terminal part of the ileum projects to a varying extent into the lumen of the large intestine as the ileal papilla. The junction between the small and large intestines occurs at the base of the papilla (Rutherford, 1926; Dyce, 1956; DiDio and Anderson, 1968, p. 152; Ellenport, 1975, p. 1552; Reeve, 1981, p. 13; Balfour, 1981, p. 193). Amongst the mammals which have been studied the papilla appears to be especially well-developed in man, dog and pig, but poorly developed in the horse.

In man the terminal part of the ileum opens into the lumen of the large intestine at the junction between the caecum and the colon. In specimens embalmed with formaldehyde the ileal papilla appears to have two transverse folds of mucous membrane which are orientated horizontally, superior and inferior to the ileal orifice. The superior mucosal fold is large, protrudes into the caecum for about 1.5 cm, and is attached to the area where the ileum joins the colon. The inferior mucosal fold is smaller, protrudes into the caecal lumen for about 0.5 cm, and is attached to the area of junction of the ileum with the caecum. The folds fuse together at their lateral ends (DiDio and Anderson, 1968, p. 154; Williams and Warwick, 1980, p. 1352; Reeve, 1981, p. 13). When the ileal papilla is examined in the living individual by endoscopy, it appears as a smooth reddish projection, elliptical or hemispherical in shape, about 1.5-2.0

cm in diameter, and projecting about 1 cm above the pink, folded mucosa of the caecum (Balfour, 1981, p. 194).

In the horse the terminal part of the ileum at the ileo-caecal orifice projects partially into the caecal lumen and is surrounded by a fold of mucous membrane. Within the fold is a venous network which distends the annular fold when engorged (Nickel et al., 1973, p. 187). This has the effect of increasing the protrusion of the ileal papilla slightly and narrowing the opening of the ileum so that the caecal contents are prevented from refluxing into the ileum. According to Nickel et al. there is no sphincter muscle at the end of the ileum. Another slit-like orifice is present between the caecum and colon and is situated beneath the ileo-caecal orifice from which it is separated by a mucosal fold (Sisson, 1975, p. 486). In the ox the ileum opens obliqely into the large intestine at the junction between the caecum and colon. The ileo-caecal orifice is located on the ileal papilla which is formed by the mucous membrane and the ileal muscle. It protrudes slightly into the lumen of the large intestine (Habel, 1975, p. 907). In the pig the ileum projects considerably into the lumen of the caecum forming a well-developed papilla (Sisson, 1975, p. 1538; Nickel et al. 1973, p. 140). According to Nickel et al. the ileal papilla in the pig is 2-3 cm long and contains circular muscle which is twice as thick as in the other parts of the ileum. The borders of the papilla are connected to the wall of the colon by mucosal folds. In the dog the junction of the ileum with the large intestine differs from the other mammalian species investigated in that the ileal papilla

projects directly into the colon forming a more or less continuous tube. The caecum opens into the colon distal to the ileo-colic junction (Dyce et al., 1987, p. 423; Evans and deLahunta, 1988, p. 196).

(b) Light microscopy.

Histological examination of the ileo-caecal sphincter in man shows that the ileal aspect of the sphincter is covered with mucosa carrying villi typical of the small intestine, whilst the caecal aspect of the sphincter is covered with non-villous mucosa typical of the large intestine (Reeve, 1981, p. 13). In the well-developed papilla of man, dog and pig the circular muscle of the terminal part of the ileum is continued into the papilla forming a thick ring at its base (Rutherford, 1926; DiDio and Anderson, 1968, p. 158; Ellenport, 1975, p. 1552). In the horse in which the ileal papilla is not clearly developed the circular muscle also forms a thick ring at the ileo-caecal orifice (Sisson, 1975, p. 486).

(4) Summary.

The most obvious sign that an anatomical sphincter exists in the gut is thickening of the circular muscle. An example of this is the pyloric sphincter of the man. However, it has been found that other tissues may take part in the formation of a sphincter. Thus, mucosal folds containing muscularis mucosae,

circular muscle or a well-developed venous plexus have been described at the ileo-caecal junction of the horse. These folds through their contractile elements could narrow the lumen of the gut at the region of the junction. Possibly the muscle or venous network may be used to maintain a constant active tone since without them a mucosal fold alone would be too weak to withstand any substantial strain.

The muscle at the junction may be thickened enough to be seen externally as a ridge or groove and this thickening may be either localised or diffuse and result in constriction of the lumen of the junction. This is seen, for example, at the gastro-oesophageal junction of the rabbit and pig. It has also been found that the position of the thickened muscle varies in different junctions and in different species, sometimes lying at the junction, sometimes being before the junction, and sometimes occurring after the junction. Not all junctions are characterised by thickening of the muscle. Thus at the gastro-oesophageal junction of the man there is no thickening of the muscle although a sphincter-like arrangement is present in the form of muscle which descends obliquely or spirally from the oesophagus through the junction into the stomach. This oblique muscle could act as a constrictor. Thus a sphincter is not always a simple annular muscle since constriction can be produced by oblique interacting muscle fibres. One feature of sphincters is that the muscle need not always be continuous between the two sides of the junction. This is seen, for example, at the pyloric sphincter of man which is formed entirely by the gastric muscle and is separated from the

duodenal muscle by a connective tissue septum. In some species such as guinea-pig this separation is not complete. Tissues other than muscle and mucosal folds, including collagenous connective tissue and elastic tissue, may be involved in the formation of the sphincter, although no thickening of these tissues has been observed apart from the the connective tissue septum at the pyloric sphincter.

Recently morphometric data on the density of innervation of the circular muscle has been found to supply information on the existence of an anatomical sphincter. This technique has not been widely used and has only been applied to junctions with obviously thickened muscle. Thus the usefulness of the method in identifying sphincters at junctions where the muscle is not grossly thickened has not been established. It seems in addition to the thickening of the muscle at the junction the increase in the density of innervation may play a very important role in controlling the passage across the junction.

III. OBJECTIVES

The overall objective of the present study is to investigate the presence of sphincters at the ileo-caeco-rectal junction and recto-coprodeal junction in the large intestine of the domestic duck (Anas platyrhynchos). The detailed objectives are as follows.

(1) To make gross observations on the internal surface anatomy, the muscle layers and the innervation of the junctions.

(2) To study the arrangement of the muscle layers at the junctions using light and transmission electron microscopy, and in the case of the ileo-caeco-rectal junction by the construction of 3-D models of the circular muscle and by scanning electron microscopy.

(3) To study the ultrastructure of the muscle cells at the junctions and compare the ultrastructure with that of muscle cells in adjacent regions of the gut.

(4) To measure the size of the muscle cells at the junctions by estimating their length and volume and compare the size with that of muscle cells in adjacent regions of the gut.

(5) To study the ultrastructure of the nerve bundles in the muscle layers at the junctions and compare the ultrastructure with that of nerves in adjacent regions of the gut.

(6) To provide quantitative data on the density of the innervation of the circular muscle by estimating the number of nerve bundles and axon profiles, and the percentage of vesiculated axon profiles and comparing the density with that in adjacent regions of the gut.

IV. MATERIALS AND METHODS

All birds used in this study were female adult domestic ducks (Anas platyrhynchos) obtained from the Tweed Valley poultry company. The birds weighed 1-1$\frac{1}{2}$ kg and ranged in age from 1$\frac{1}{2}$-2 years.

A. ILEO-CAECO-RECTAL JUNCTION.

(1) Gross Observations.

Twelve ducks were used to study the gross structure of the ileo-caeco-rectal junction. Six birds were preserved by immersion in 10% formal saline. The remaining six birds were unfixed. The region of the ileo-caeco-rectal junction was removed from the birds and washed gently with saline solution to get rid of ingesta. The intestinal tract at the junction was cut into two halves. The gross structure of the ileo-caeco-rectal junction was investigated with the aid of a Nikon dissecting microscope.

The innervation of the large intestine was studied in ten birds. Five birds were fixed by immersion in 10% formal saline. The other five birds were unfixed. The nerves were investigated with the aid of a Nikon dissecting microscope.

(2) Histological Study of the Musculature of the Ileo-Caeco-Rectal Junction.

(a) Light microscopy.

The histology of the ileo-caeco-rectal junction was investigated in 20 birds. Birds were killed by an overdose of pentobarbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight), and immediately after death the ileum, caecum and rectum were removed from the birds and fixed in either 10% formal saline for seven days (10 birds) or Bouin's fluid for 24 hours (10 birds). The intestine in some specimens (10 birds) was ligated 2.5 cm on either side of the junction, injected with fixative in order to obtain a moderate distension of the wall, and then suspended in the fixative solution. The degree of distension was the same in the different specimens, the injection pressure always being about 10 mmHg. In other specimens (10 birds) fixation was achieved by simple immersion in fixative. Following fixation the specimens were trimmed to a length of about 1.5cm on either side of the junction. The tissue was routinely dehydrated, infiltrated and embedded in Paraplast Plus (M.P. 55-57 ^{o}C) with the help of a Shandon tissue processor. Serial sections cut transversely and longitudinally to the length of the gut were prepared at 7 μm thickness using an American Optical rotary microtome. Alternate sections were stained with haematoxylin and eosin and Masson's trichrome (Culling, 1974). Photomicrographs were prepared using a

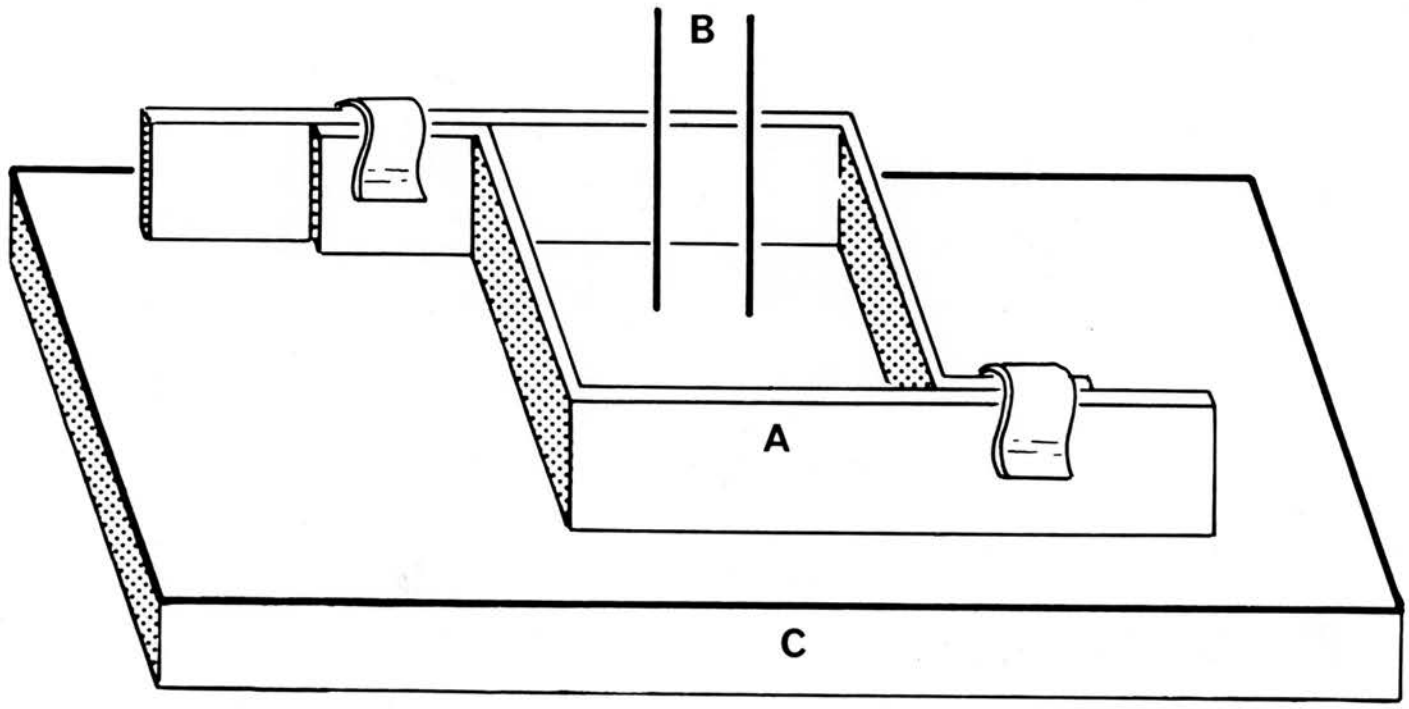
Leitz-Orthoplan microscope and a Leitz Vario Orthomat 2 camera.

(b) 3-D reconstruction of the circular muscle layer.

Material for 3-D reconstruction was taken from two adult ducks. The intestine of one specimen was ligated at about 2.5 cm on either side of the ileo-caeco-rectal junction and injected with Bouin's fluid (injecton pressure about 10 mmHg) in order to obtain a moderate degree of distension. The specimen was then left in the same solution. Fixation of the other specimen was by immersion in Bouin's fluid.

For reconstruction, it was necessary to introduce extrinsic reference points in the Paraplast Plus embedding medium. The method used was that of Langemeijer and Simon (1973) with slight modifications. The modifications consisted of vertically positioning in the mould the steel pins used to introduce the reference points rather than the horizontal position adopted by Langemeijer and Simon. This facilitated the positioning and orientation of the tissue in the mould in relation to the pins. Furthermore, two pins were used instead of three in order to reduce to a minimum distortion in the tissue caused by handling and processing. A brass embedding mould was placed on a thick piece of rubber about 25 mm in thickness and was perforated vertically by two parallel steel pins (Fig. 1). Each pin had a diameter of about 1 mm and was fixed as close to the embedded tissue as possible in order to avoid losing the reference points

Fig. 1. Drawing of the brass embedding mould (A) used in the preparation of the 3-D reconstruction model. The specimen is placed in the mould close to two steel pins (B) which are vertically positioned in the mould and used to introduce the reference points. The pins perforate a thick rubber pad (C) on the top of which the mould is placed.


B
A
C

when the block needed to be trimmed. The specimen was placed in the mould close to the two pins and the mould was then filled with Paraplast Plus (M.P. 55-57 $^{\circ}C$) and left to harden gradually. When the block becomes hard enough the two pins were removed carefully leaving two parallel empty canals.

These two canal were filled with a paraffin-charcoal mixture which was prepared by mixing and stirring together equal parts of paraffin (M.P. 40-42 $^{\circ}C$) and charcoal and placing the mixture in an oven at 50 $^{\circ}C$. The paraffin-charcoal mixture was injected slowly and carefully into the two canals using a syringe kept at the same temperature as the mixture. The Paraplast block was then left to set at room temperature which allowed the two bars of the paraffin-charcoal mixture to harden. After that the block was placed in the microtome and serial transverse sections at 15μm thick were cut. The sections were mounted on glass slides using an albumen-glycerin solution. The slides were left in the incubator at 37 $^{\circ}C$ and the temperature then raised slightly above the melting point of the paraffin used in the mixture. This caused the paraffin to melt leaving the charcoal particles adhering to the slide. The two charcoal areas close to the tissue sections served as landmarks (Fig. 2).

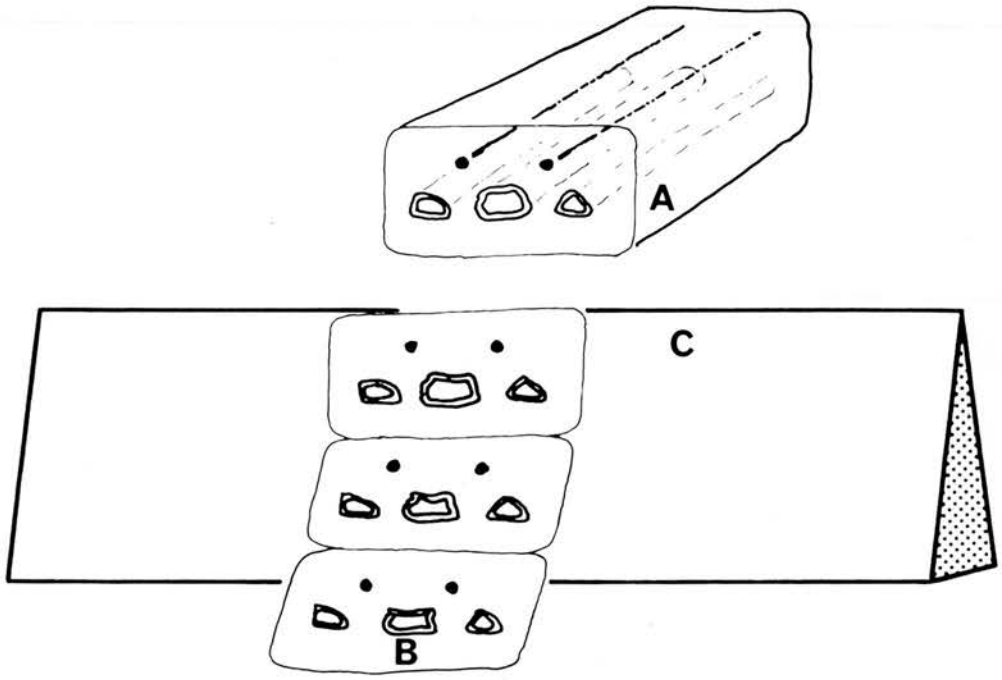
The ileo-caeco-rectal junction was reconstructed following the photographic technique method of Los (1970). This included serial photography of every third transverse microscopical section on 35 mm negative film. To achieve this only a low-power lense (X10) was required. The negatives were then enlarged

Fig. 2. Drawing to show the histotechnical procedure used in the preparation of the 3-D reconstruction model.

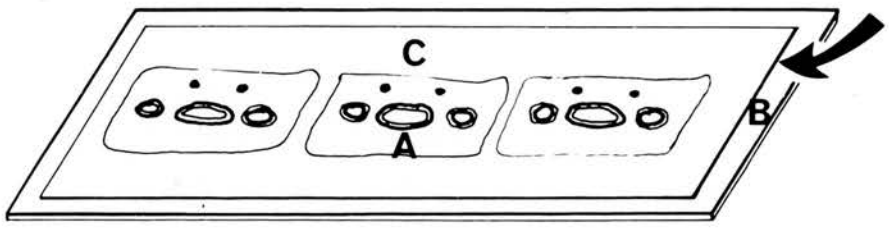
In (a) the Paraplast Plus block (A) containing the tissue and two parallel bars is placed in the the microtome and serial transverse sections (B) are cut with the knife of the microtome (C).

In (b) the tissue sections (A) are mounted on a glass slide (B) using a thin layer of albumen- glycerine solution (C).

a



b



nine times using the photographic enlarger and printed. The appropriate thickness was obtained by fixing cardboard, 1.15 mm thick, to the back of each print so that a total thickness (which included 0.20 mm of the thickness of the photographic paper and glue) of 1.35 mm was obtained.

The circular muscle layer was then cut out and the trimmed photographs arranged in series. The position of the prints in the reconstruction was determined by the reference points and by the positions of the lumens of the ileum, caeca and rectum. By using this technique the whole circular muscle layer of the ileo-caeco-rectal junction was reconstructed.

(3) Ultrastructure of the Muscle Cells and Nerve Bundles in the Region of the Ileo-Caeco-Rectal Junction.

(a) Scanning electron microscopic observations of the musculature.

To study the arrangement of the musculature and to make a montage of the muscle layer of the ileo-caeco-rectal junction specimens were removed from 16 adult ducks and fixed with 3% glutaraldhyde in 0.1M sodium cacodylate at pH 7.3 for three hours at room temperature. The tissues were then processed by the modified tannic acid method of Murakami et al. (1977) in which the specimens were left overnight in a solution of 2% guanidine hydrochloride and 2% tannic acid in water. After several washings in distilled water, the tissues were post-

fixed in 2% aqueous osmium tetroxide for eight hours. Following dehydration in a graded series of acetone (50%, 70%, 90% and 3 X 100%) for 30 minutes in each solution, the tissue was then critical-point dried by immersion in liquid carbon dioxide (Polaron C.P.D.) (Anderson, 1951). The specimens were then glued to an aluminium stub with conductive carbon cement and sputtered with 20 nm gold/palladium (40:60) in an EM Scope SC 500 sputter coater (Panayi et al., 1977). Specimens were then viewed and photographed in a Philips SEM 505 scanning electron microscope using a beam-accelerating voltage of 20-30kv. Scanning electron micrographs were made on Ilford 35 mm Fp4 black and white film.

(b) Transmission electron microscopic observations of the muscle cells and nerve bundles.

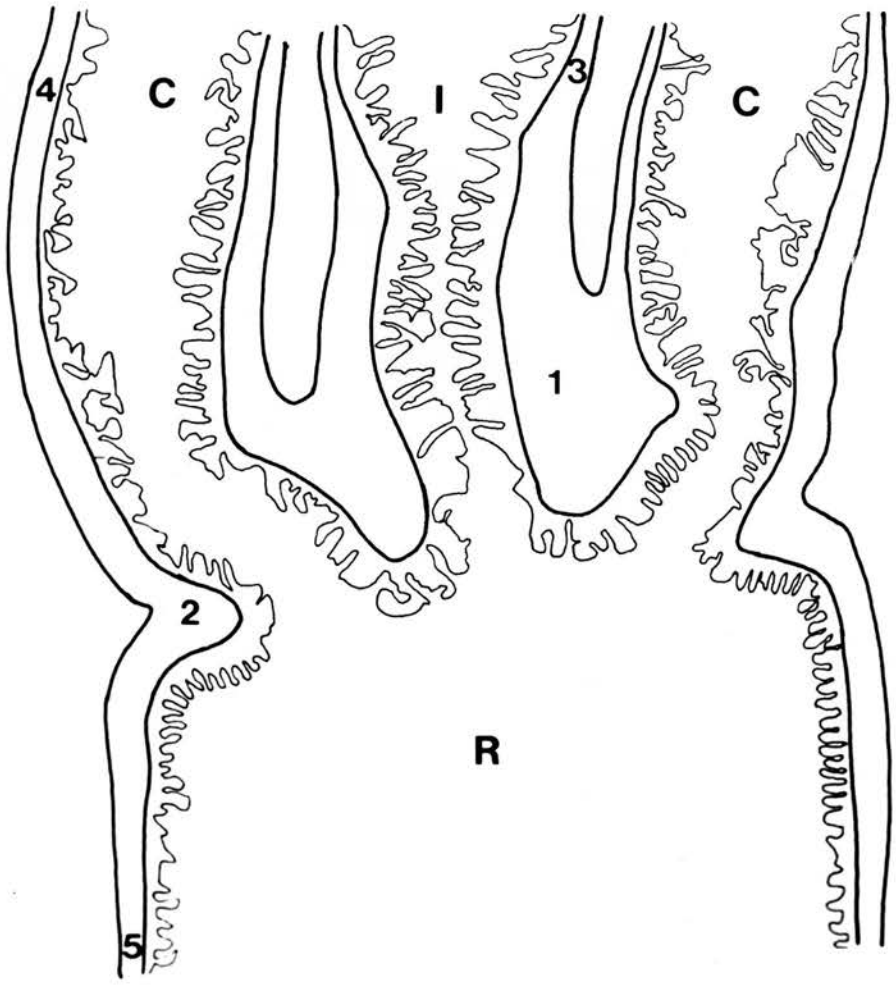
Specimens from 20 adult ducks were taken under pentobarbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight). The abdomen of the birds was opened and the ileo-caeco-rectal junction was removed and trimmed under the fixative solution into rings 1-2 mm in length. The specimens were fixed with 6% glutaraldhyde in 0.1M sodium cacodylate buffer solution (Sabatini et al., 1963) at pH 7.3 for two and a half hours at room temperature. After several washings with cacodylate buffer solution the tissues were post-fixed with 1% osmium tetroxide in 0.1M cacodylate buffer solution (Palade, 1952) at pH 7.3

for about one hour at room temperature. Following dehydration in a graded series of acetone solutions, the tissue was infiltrated and embedded in araldite mixture. In order to orientate and select an area for the ultrastructural observations semi-thin sections 1 μm in thickness were cut with glass knives, stained with 1% toluidine blue and examined under the light microscope. Ultra-thin sections with silver gold interference colour (approximately 70-80 nm in thickness) were cut on an OMU4-Reichert Ultracut ultramicrotome and collected on 200 mesh uncoated copper grids and on coated grids with 1.5% low viscosity nitrocellulose (Parlodion) 20 nm in thickness. The sections were double stained in saturated uranyl acetate in 50% methanol for 30 minutes followed by lead citrate in water (Hayat, 1970) for 5 minutes. The areas selected for investigation were the base of the ileal papilla, around the orifices of the caeca and the ileum, and the caecum and rectum 5 mm from the ileo-caeco-rectal junction (Fig. 3). The grids were examined and photographed in a Philips EM 400 electron microscope.

(4) Quantitative Observations on the Musculature in the Region of the Ileo-Caeco-Rectal Junction.

The muscle cell length and volume at the region of the ileo-caeco-rectal junction were calculated in three adult ducks. Tissue was taken under pento-

Fig. 3. Drawing of a light micrograph of the ileo-caeco-rectal junction. The quantitative study of the nerve bundles in the circular muscle layer was carried out at the base of the ileal papilla (1); at the caecal orifice (2); and in the ileum (3); caecum (4) and rectum (5) 5 mm from the junction. C, caecum; I, ileum; R, rectum. X 11.


4
C
I
3
C
1
2
R
5

barbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight). Immediately after removal from the body, the specimens were immersed in a fixative solution of 6% glutaraldhyde in 0.1M sodium cacodylate buffer at pH 7.3 and trimmed into rings 1-2 mm long. The specimens were then immersed in a fresh fixative solution for two and a half hours at room temperature. Dehydration, embedding, cutting and staining of the grids were the same as in the transmission electron microscopic observations of the muscle cells and nerve bundles described on page 33.

(a) Muscle cell length.

For the measurement of muscle cell length in the muscularis mucosae, circular muscle layer and longitudinal muscle layer at the ileo-caeco-rectal junction, sections transverse to the thickness of the wall and parallel to the length of the gut were photographed in the electron microscope at X 2750 and prints at a magnification of X 5500 were prepared. The percentage of the muscle cell profiles showing nuclei was counted. The average length of the muscle cell nuclei was measured in semi-thin 1 um thick longitudinal sections cut transversely to the length of the gut. Both transverse and longitudinal sections were cut from the same block. The measurement of the muscle cell length was based on the formula used by Gabella (1976) shown below.

$$\text{cell length} = \frac{\text{nucleus average length X 100}}{\text{\% of nucleated cell profiles}}$$

(b) Muscle cell volume.

For the calculation of the volume of the muscle cells of the muscularis mucosae, circular muscle layer and longitudinal muscle layer, the surface area of the muscle cell profiles was measured in the electron micrographs of the transverse sections of the muscle cells using a Reichert-Jung MOP-Video Plan. The total number of the nucleated muscle cell profiles was also counted. The measurement of the muscle volume was based on the formula used by Gabella (1976) shown below.

$$\text{cell volume} = \frac{\text{sum of all profile surfaces X nucleus length}}{\text{number of nucleated profiles}}$$

(5) Quantitative Observations on the Innervation in the Region of the Ileo-Caeco-Rectal Junction.

For the quantitative study of the nerve bundles, the total number of axon profiles and vesiculated and non-vesiculated axon profiles was counted in ten birds. Under pentobarbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight) the ileo-caeco-rectal junction was removed and cut into rings 1-2 mm in length. The five areas which were investigated are shown in Figure 3 . These were the base of the ileal papilla, around the orifices of the caeca and ileum, and the caecum and rectum 5 mm from the ileo-caeco-rectal junction. The fixation, dehydration, embedding, cutting and staining of the grids were the same as described on page 33 for the transmission electron microscopic observations of muscle cells and nerve bundles.

Large areas of the circular muscle layer were photographed in the electron microscope at X 2750 and prints at a magnification of X 5500 were assembled into a photographic montage. Each intramuscular nerve bundle in the montage was rephotographed at a magnification of X 10,000 and final prints were prepared at a magnification of X 20,000. The data obtained from the montages included the number of nerve bundles and axon profiles and the percentage of non-vesiculated and vesiculated axon profiles per number of circular muscle cell profiles. These data were subjected to statistical analysis using Student's "t test".

B. RECTO-COPRODEAL JUNCTION.

(1) Gross Observations.

The caudal part of the large intestine and the cloaca, including the region of the recto-coprodeal junction, were removed from 12 adult ducks and washed gently with normal saline solution. Specimens from four birds were ligated at about 2.5 cm on either side of the junction and injected with 10% formal saline fixative solution and then suspended in the solution. Four of the remaining eight birds were fixed by simple immersion in the fixative solution whilst the other four birds were examined unfixed. The specimens were then trimmed and cut longitudinally into two halves. The examination of the specimens was carried out with the aid of a Nikon dissecting microscope.

(2) Histological Study of the Musculature of the Recto-Coprodeal Junction.

Specimens were removed from 14 adult ducks. The birds were killed by an overdose of pentobarbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight), and the caudal part of the large intestine and the cloaca were removed

from the body immediately following death. The specimens were then washed with saline solution, ligated at about 2.5 cm on either side of the junction and gently distended with the fixative solution (injection pressure always being about 10 mmHg), and then suspended in the solution. Tissue from seven birds was fixed with 10% formal saline for seven days while the specimens from the other seven birds were fixed with Bouin's fluid for 24 hours. Following fixation the tissues were trimmed to about 1.5 cm on either side of the junction, processed routinely and finally embedded in Paraplast Plus (M.P. 55-57 $^{\circ}C$). 7 μm thick serial sections were cut transversely and longitudinally to the length of the gut using an American Optical rotary microtome. The sections were stained with either haematoxylin and eosin or Masson's trichrome (Culling, 1974).

(3) Ultrastructure of the Muscle cells and Nerve Bundles in the Region of the Recto-Coprodeal Junction.

For transmission electron microscopy specimens were removed from 12 adult ducks under pentobarbitone anaesthesia (Sagatal) (60 mg/ 5 lb body weight), immediately immersed in the fixative solution of 6% glutaraldhyde in 0.1M sodium cacodylate buffer solution at pH 7.3 and cut into rings 1-2 mm in length. The tissue was then transferred into a fresh fixative solution for two and a half hours at room temperature. The four areas investigated are shown in Figure 4.

These were the middle of the rectum, the rectum and coprodeum 5 mm from the recto-coprodeal junction, and the recto-coprodeal junction. Dehydration, embedding, cutting and staining of the grids were the same as described on page 33. Sections were viewed and photographed in a Philips EM 400 electron microscope.

(4) Quantitative Observations on the Musculature in the Region of the Recto-Coprodeal Junction.

The length and volume of the muscle cells at the recto-coprodeal junction were calculated in three adult ducks. The specimens were removed from the birds under pentobarbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight) and trimmed under the fixative solution into rings 1-2 mm in length. The fixation, dehydration, embedding, cutting and staining of the grids were the same as described on page 33.

(a) Muscle cell length.

For the measurement of muscle cell length in the muscularis mucosae, circular muscle layer and longitudinal muscle layer, semi-thin 1 μm thick longitudinal sections cut transversely to the length of the gut were stained with 1% toluidine

blue. The average length of the muscle cell nuclei was counted. From the same block ultra-thin transverse sections cut parallel to the length of the gut were photographed in the electron microscope to calculate the percentage of the nucleated muscle cell profiles. The same formula adopted for the measurement of the muscle cell length at the ileo-caeco-rectal junction and described on page 36 was used.

(b) Muscle cell volume.

The volume of the muscle cells of the muscularis mucosae, circular muscle layer and longitudinal muscle layer was counted in transverse ultra-thin sections by measuring the surface area of the muscle cell profiles using a Reichert-Jung MOP-Video Plan and counting the total number of the nucleated muscle cell profiles. The same formula adopted for the measurement of the muscle cell volume at the ileo-caeco-rectal junction and described on page 36 was used.

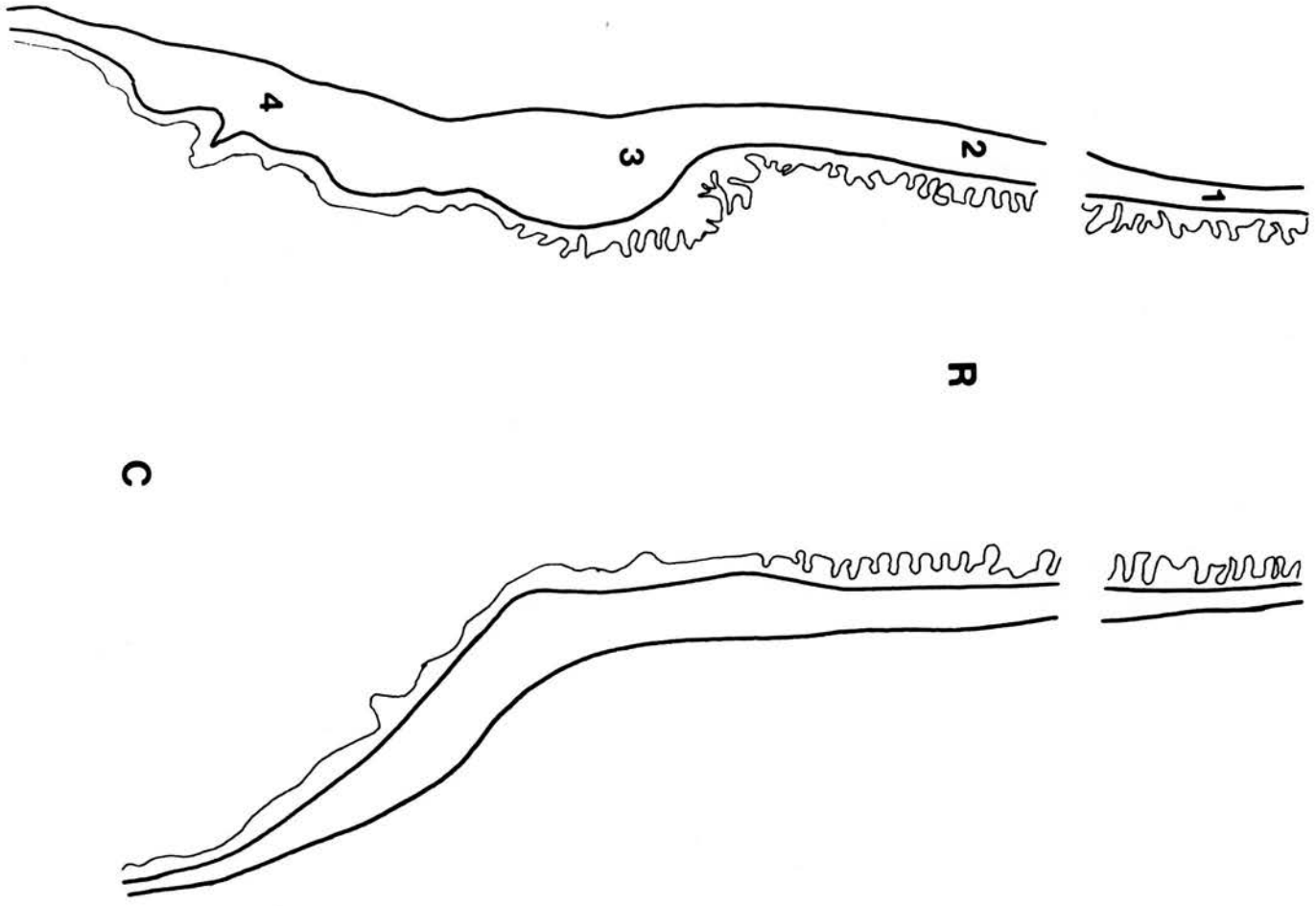
(5) Quantitative Observations on the Innervation in the Region of the Recto-Coprodeal Junction.

The number of nerve bundles and the total number of axon profiles and the percentage of non-vesiculated and vesiculated axon profiles were counted in 10

birds. The specimens were removed from the body of the birds under pento-barbitone sodium anaesthesia (Sagatal) (60 mg/ 5 lb body weight) and cut into rings 1-2 mm in length. The areas examined were the middle of the rectum, the rectum and coprodeum 5 mm from the recto-coprodeal junction, and the recto-coprodeal junction (Fig. 4). The fixation, dehydration, embedding, cutting and staining of the grids were the same as in the transmission electron microscopic observations of the muscle cells and nerve bundles described on page 33.

The photographic montages of the circular muscle cells were prepared by photographing transverse ultra-thin sections in the electron microscope using the method identical to that in the quantitative study of the nerve bundles at the ileo-caeco-rectal junction described on page 37. The data obtained from these montages included the number of nerve bundles, the total number of axon profiles, and the percentages of non-vesiculated and vesiculated axon profiles per number of circular muscle cell profiles. These data were subjected to statistical analysis using Student's "t test".

Fig. 4. Drawing of a light micrograph of the recto-coprodeal junction. The quantitative study of the nerve bundles in the circular muscle layer was carried out in the middle portion of the rectum (1), in the rectum (2) and coprodeum (4) 5 mm from the junction, and close to the recto-coprodeal junction (3). C, coprodeum; R, rectum. X 12.


4
3
2
1
R
C

V. OBSERVATIONS

A. ILEO-CAECO-RECTAL JUNCTION.

(1) Gross Observations.

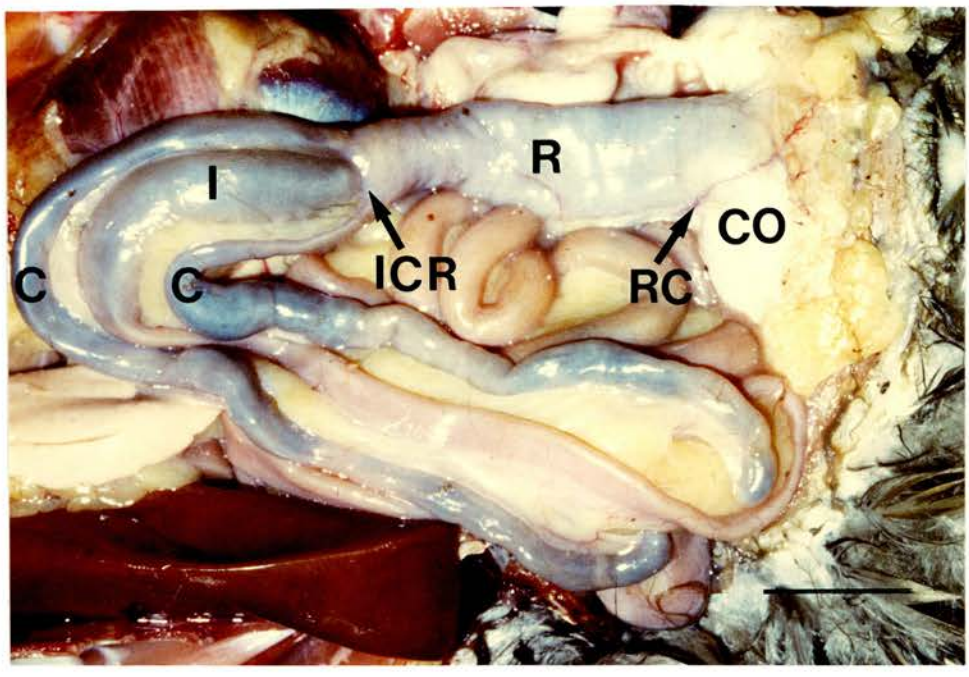
The terminal part of the ileum was funnel-shaped, its diameter gradually decreasing caudally. 1.5 mm proximal to the ileo-rectal orifice the lumen of the ileum was about 1.6-2 mm in diameter. The diameter slightly increased caudally until at the ileo-rectal orifice it was about 2- 2.4 mm in diameter. At the junction with the rectum the ileum projected into the rectal lumen for a distance of about 1.5-2 mm. However, this projection was not equally developed, the dorsal part being shorter than the ventral part. The lumen of the rectum had a uniform diameter of about 6.5-7 mm which was considerably greater than that of the ileum. On either side of the ileal projection and ventrolateral in position were the openings of the large, dark-green right and left caeca (Fig. 5). Each caecum at its base had a smaller diameter than the terminal part of the ileum, the diameter around the caecal orifices being about 1.4-1.8 mm.

(2) Nerve Supply of the Large Intestine.

(a) Splanchnic nerves.

The nerves which contributed branches to the intestinal nerve at the ileo-

Fig. 5. The gross anatomy of ventral view of the large intestine. The ileum (I) joins the cranial end of the rectum (R) close to the point where the right and left caeca (C) arise from the rectum. The caudal end of the rectum joins the coprodeal compartment (CO) of the cloaca. ICR, ileo-caeco-rectal junction; RC, recto-coprodeal junction. Scale, 1 cm.

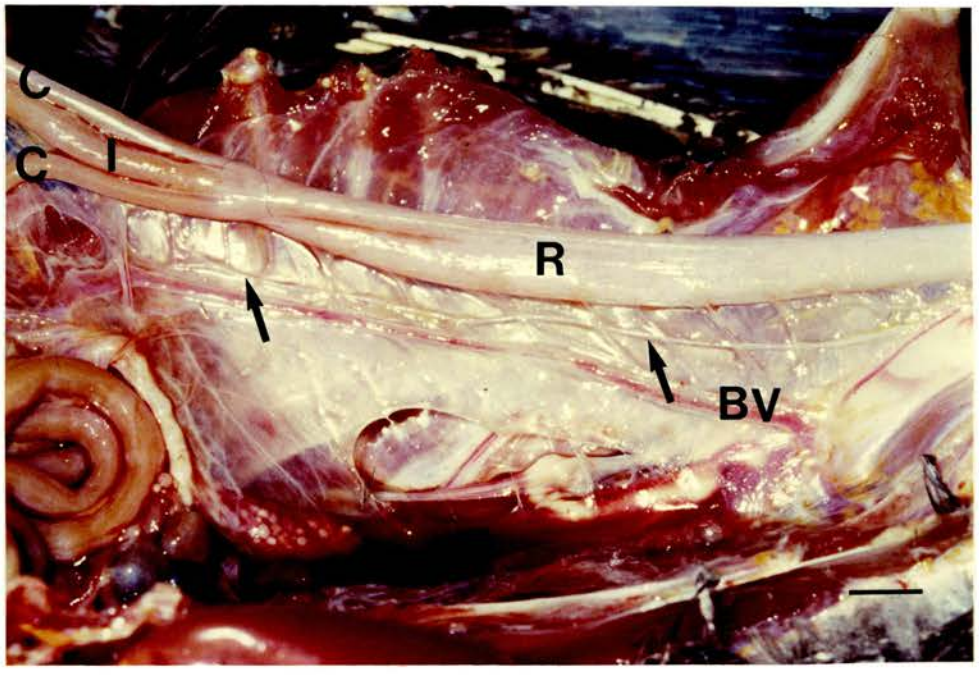

I
R
CO
ICR
RC
C
C

caeco-rectal junction were the caudal splanchnic nerves. They were derived from the eighth, ninth, tenth and eleventh synsacral ganglia and were united together to form a single nerve which ran caudally in the mesentery close to the ventral aspect of the aorta. From the ventral side of this nerve many small branches passed ventrally in the mesentery to join the intestinal nerve. The nerve ended by dividing into two branches. One small branch curved ventrally to join the intestinal nerve in the region of the recto-coprodeal junction. The other branch continued caudally to join the caudal plexus

(b) Intestinal nerve.

Many branches of the intestinal nerve (Fig. 6) descended in the mesentery at regular intervals to end in the wall of the intestine. The intestinal nerve was a large trunk originating from a plexus lying between the coeliac and cranial mesenteric arteries. It extended caudally close to and parallel with the intestine from the duodenum to the cloaca. Throughout its course it received many branches from the cranial mesenteric, caudal mesenteric and aortic plexuses as well as thin rami from the synsacral and caudal splanchnic nerves. The ganglia on the intestinal nerve were so small that they were not observed macroscopically. Only 1-2 large ganglion was found in the region of the ileo-caeco-rectal junction. The intestinal nerve increased considerably in size in the region of the

Fig. 6. The intestinal nerve (arrows) runs in the mesentery between the intestine and the blood vessele (BV) and gives many branches to the wall of the gut. C, caecum; I, ileum; R, rectum. Scale, 1 cm.


C
C
I
R
BV

junction between the small and large intestines and continued to be large on its course along the rectum. At the recto-coprodeal junction the intestinal nerve joined a nerve formed by the caudal splanchnic nerves.

(3) Histological Study of the Musculature of the Ileo-Caeco-Rectal Junction.

(A) Light microscopy.

The musculature in the wall of the ileum, caeca and rectum consisted of the muscularis mucosae and the muscle tunic (Fig. 7).

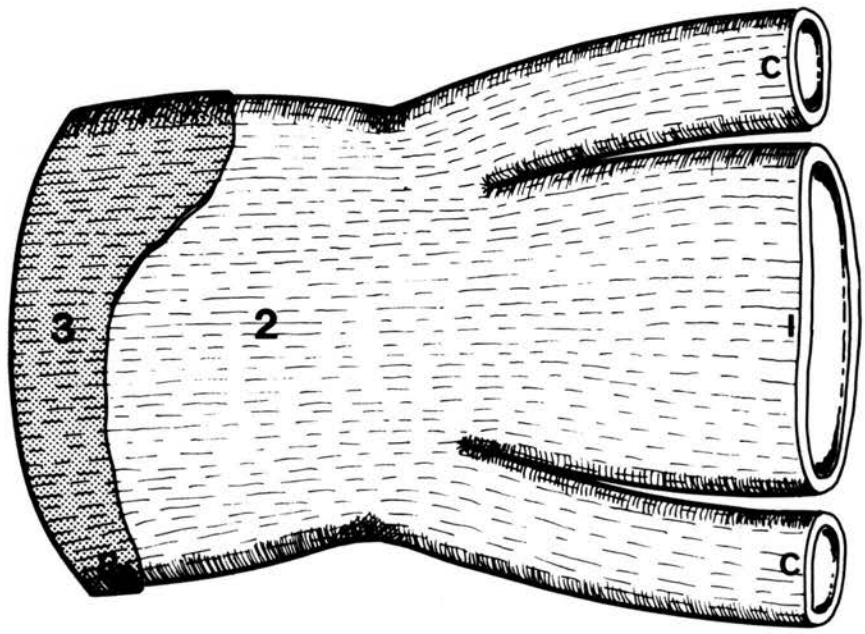
(a) Muscularis mucosae.

The muscularis mucosae at the ileo-caeco-rectal junction consisted of longitudinally orientated closely packed muscle fibres.

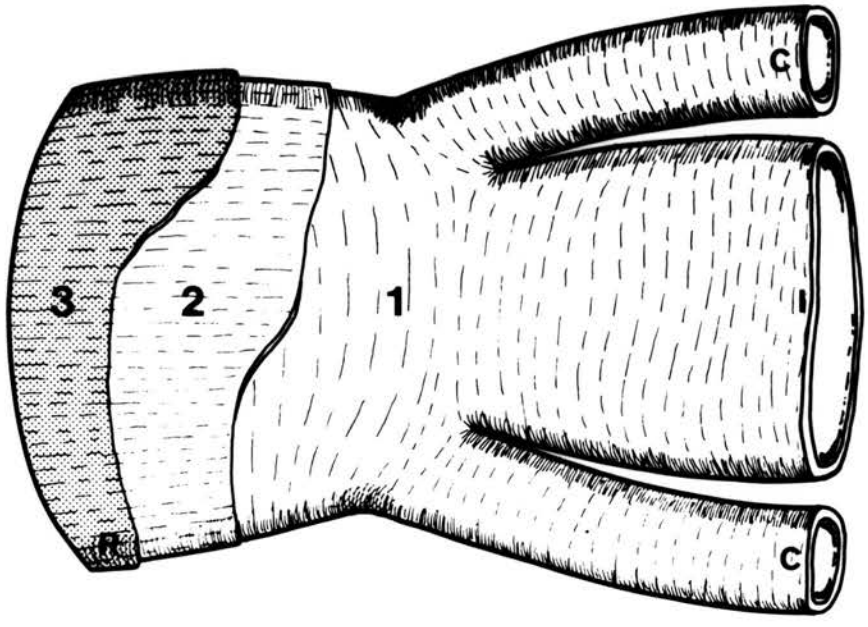
The muscularis mucosae of the ileum 5 mm from the junction with the rectum was about 30-35 μm thick. It increased gradually towards the ileo-rectal junction and at the tip of the ileal papilla became about 90-100 μm thick. It became continuous on either side of the ileal papilla at the caecal orifices with the muscularis mucosae on the medial sides of the right and left caeca.

Fig. 7. Drawings from macerated preparations showing the muscle tunics at the ileo-caeco-rectal junction. (a) after removing the serosa (3), and (b) after removing the outer longitudinal muscle layer (2). At the junction the outer longitudinal muscle layer of the ileum (I) and caeca (C) is continued caudally by the longitudinal muscle of the rectum (R). The circular muscle layer (1) forms a thick ring at the junction of the ileum and rectum and a thick ring at the origin of each caecum. X 10.

a



b



The muscularis mucosae of each caecum 5 mm from the ileo-caeco-rectal junction was very thin being only about 15-22 μm thick. It increased gradually towards the base until around the caecal orifice it became about 70-75 μm thick. It was continuous medially with the muscularis mucosae of the ileal papilla and laterally with the muscularis mucosae of the rectum.

The muscularis mucosae of the rectum 5 mm from the junction with the ileum and caeca was about 35-40 μm thick.

(b) Muscle tunic.

The muscle tunic at the ileo-caeco-rectal junction consisted of a very thick inner circular layer and a thin outer longitudinal layer. The arrangement of the muscle layers is shown in the serial transverse sections in Figures 8 and 9 and in the serial longitudinal sections in Figure 10.

(i) Longitudinal muscle layer.

The longitudinal muscle layer of the ileo-caeco-rectal junction consisted of loosely packed, irregularly arranged, muscle fibres. It was separated from the inner circular muscle layer by a very thin layer of connective tissue.

The longitudinal muscle layer of the ileum 5 mm from the junction with the

rectum was about 35-40 μm thick. It increased gradually towards the base of the ileal papilla (Fig. 9 e-h) until 1.5-2 mm cranial to the ileo-rectal orifice it became about 150-160 μm thick. Here it was continuous caudally with the longitudinal layer of the rectum. Immediately proximal to the base of the ileal papilla the longitudinal muscle layer became continuous with the longitudinal layers of the right and left caeca (Fig. 10 e-g). The longitudinal muscle layer did not extend into the ileal papilla.

The longitudinal muscle layer of each caecum 5 mm from the ileo-caeco-rectal junction was about 20-25 μm thick. Its width increased gradually towards the base of the caecum, and around the caecal orifice it was about 100-112 μm thick. Laterally the layer became continuous distally with the longitudinal muscle of the rectum, whilst medially it was continuous with the longitudinal layer of the ileum (Figs. 9 e-h; 10 h-j).

The longitudinal muscle layer of the rectum was more regular and more tightly packed than that of the ileum and caeca. 5 mm from the ileo-caeco-rectal junction it was about 45-50 μm thick.

(ii) Circular muscle layer.

The circular muscle layer of the ileum 5 mm from the junction with the rectum consisted of tightly packed muscle bundles separated by connective tis-

sue. Here it was about 310-476 μm thick. The thickness gradually increased caudally (Fig. 9 a-d) until 1.5 mm proximal to the ileo-rectal orifice at the base of the ileal papilla it was about 1007-1102 μm thick. At this point the circular muscle formed a thick ring (Figs. 9 i-k; 10 a-d) consisting of elongated, loosely packed, irregular muscle bundles which fused on either side with the medial parts of the thickened circular muscle at the bases of the right and left caeca (Fig. 10 a-d). Distal to the base of the papilla the muscle gradually thinned until at the apex of the papilla it was 680-760 μm thick. The ventral part of the ileal papilla extended approximately 100-200 μm further caudally than the dorsal part with the result that the ileal protrusion into the lumen of the rectum appeared to be asymmetrically developed (Figs. 9 l-o; 10 e-g).

The circular muscle layer of each caecum 5 mm from the ileo-caeco-rectal junction consisted of closely packed muscle bundles. Here it was about 300-385 μm thick. Towards the base of the caecum the muscle increased gradually (Fig. 9 a-d) in thickness and around the origin of each caecum it formed a ring about 870-988 μm in thickness which protruded into the lumen of the gut (Fig. 10 a-d). The ring consisted of an ovoid mass of loosely packed irregular muscle bundles. Its medial part fused with the muscular ring of the ileum whilst its lateral part was continuous caudally with the circular layer of the rectum (Fig. 10 a-d). Since each caecum joined the rectum obliquely the medial and the lateral parts of the thickened circular muscle at the caecal orifice appeared on histological sections to lie at different levels (Fig. 10 h-j).

The circular muscle layer of the rectum consisted of tightly packed, regular muscle bundles. 5 mm from the ileo- caeco-rectal junction it was about 380-446 μm thick.

(B) 3-D reconstruction of the circular muscle layer.

Two reconstructed models of the circular muscle layer of the ileo-caeco-rectal junction are shown in Figure 11 a, b. They clearly demonstrate three-dimensionally that the thickness of the circular muscle was markedly increased over the most distal 3.5 mm of the ileum and around the caecal orifice for a length of 1.5 mm. The thickened circular muscle layer at the base of the ileal papilla is shown to be fused on either side with the medial parts of the thickened circular muscle around the orifices of the right and left caeca. The thickened muscle around the caecal orifice protrudes into the lumen of the gut.

Fig. 8. Photograph of the ileo-caeco-rectal junction. The lines a-o indicate the approximate positions of the serial transverse sections in Fig. 9 . Section (a) is cranial to the junction and section (o) is caudal to the junction. C, caecum; I, ileum; R, rectum. X 10.

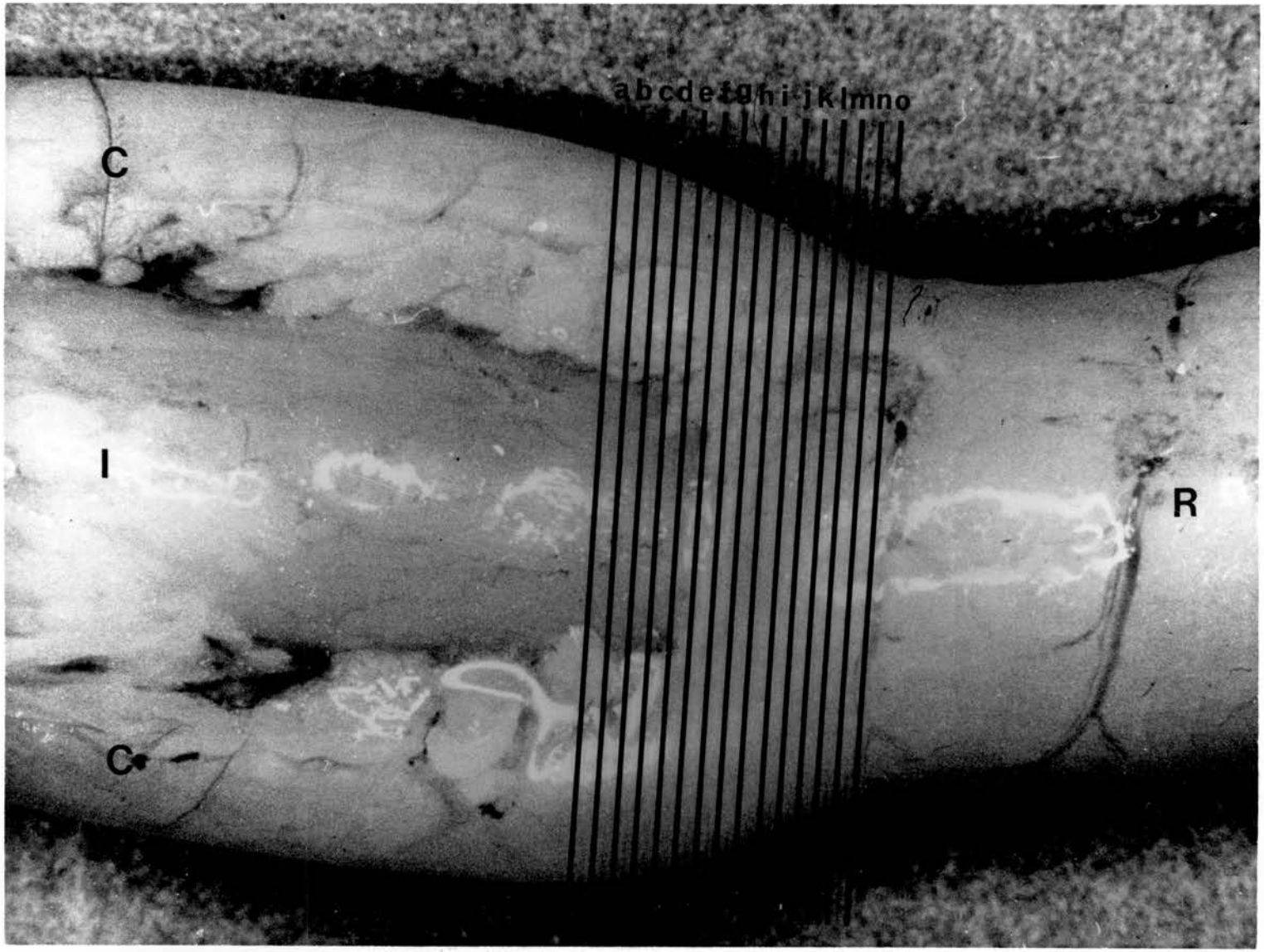
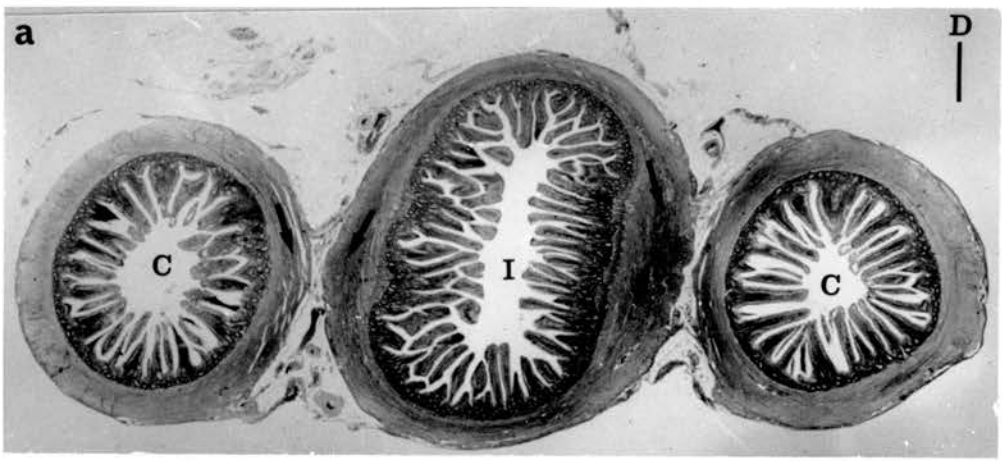

a b c d e f g h i j k l m n o
C
I
R
C

Fig. 9. Light micrographs of serial transverse sections of the ileo-caeco-rectal junction. The levels of the sections are shown in Fig. 8 . C, caecum; D, dorsal; I, ileum; IP, ileal papilla; R, rectum. Masson's trichrome stain. X 10.

a, b, c, d:

The circular muscle layer of the terminal part of the ileum and the medial parts of the caeca 5 mm from the ileo-caeco-rectal junction gradually increase in thickness and fuse together (arrows).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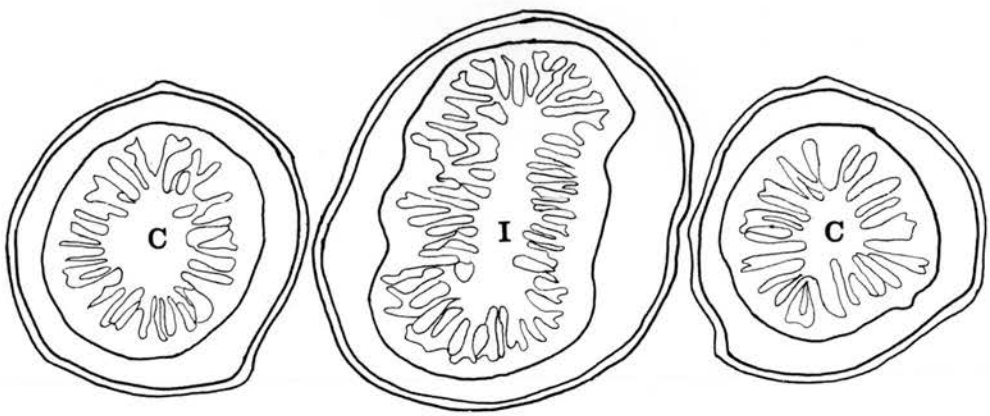
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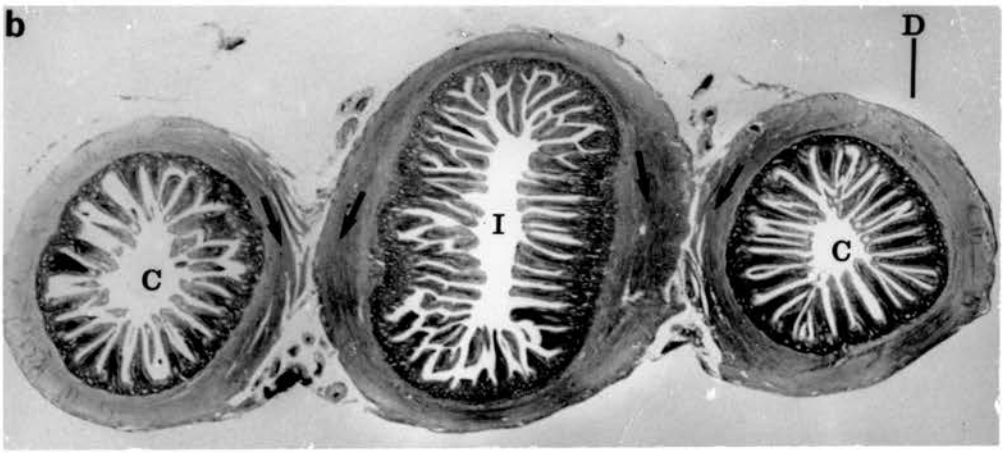
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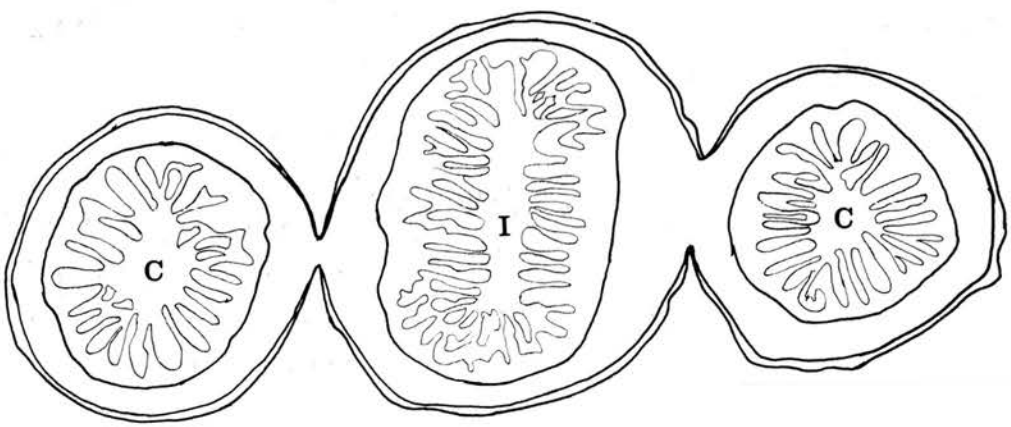
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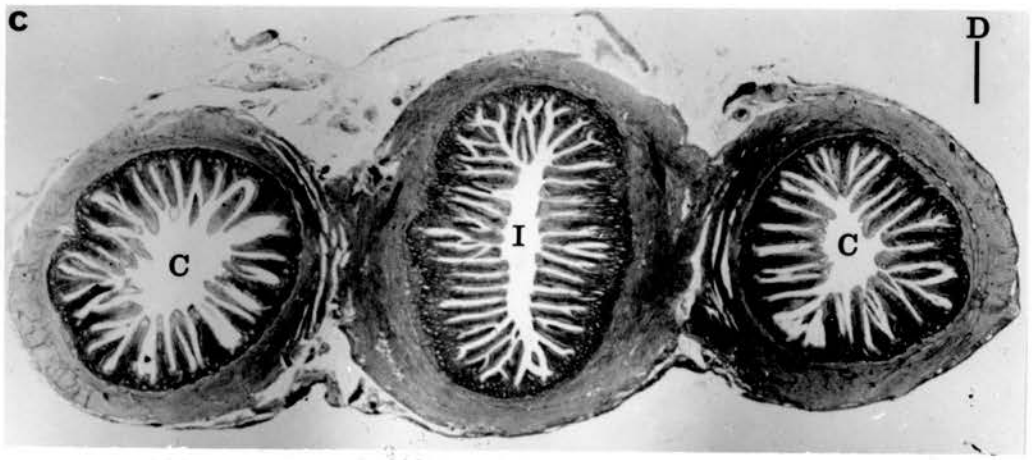
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D
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I
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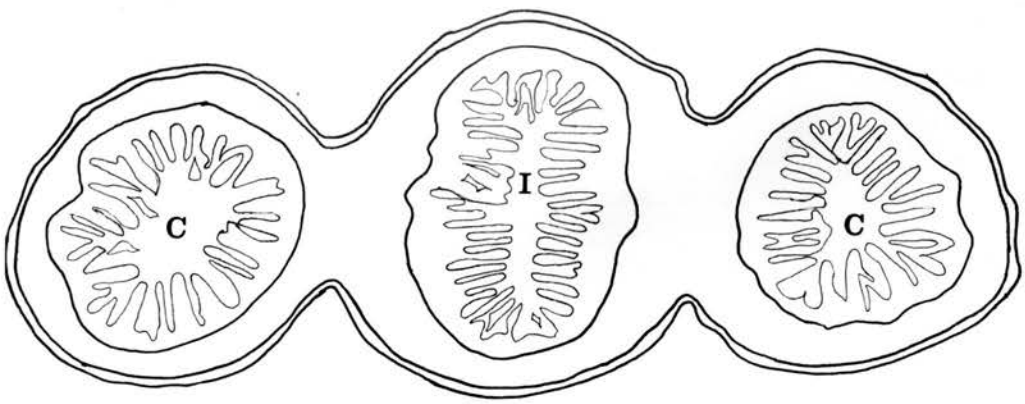
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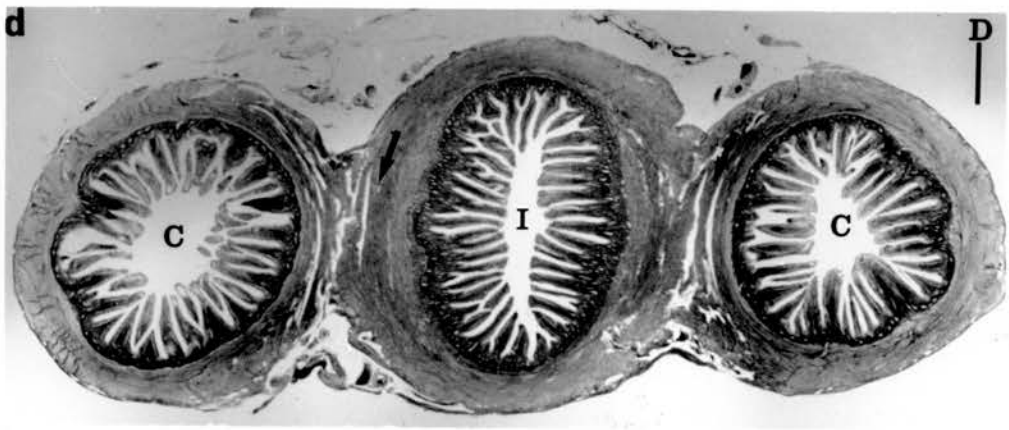
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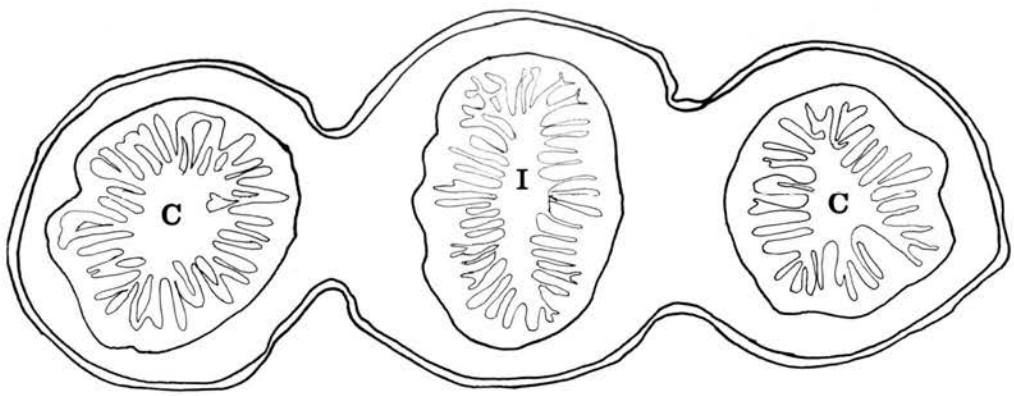
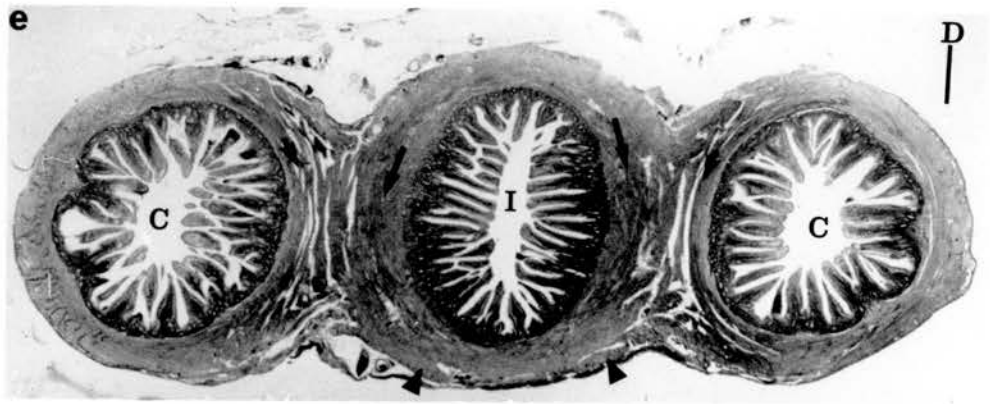

C
I
C

Fig. 9. Light micrographs of serial transverse sections of the ileo-caeco-rectal junction. The levels of the sections are shown in Fig. 8 . C, caecum; D, dorsal; I, ileum; IP, ileal papilla; R, rectum. Masson's trichrome stain. X 10.

e, f, g, h:

The circular muscle layer continues increasing in thickness towards the ileo-caeco-rectal junction forming a thick muscular ring involving the base of the ileal papilla (long arrows) and the medial walls of the two caeca (short arrows). The longitudinal muscle layer (arrowheads) increases in thickness and is continuous on either side with the longitudinal muscle layer of the right and left caeca.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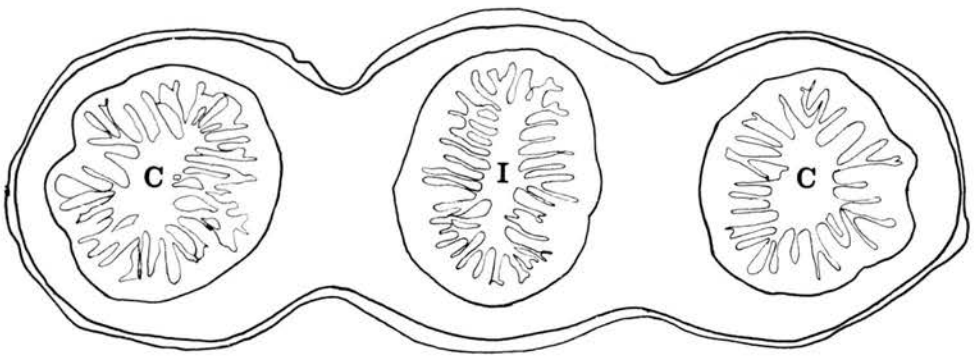
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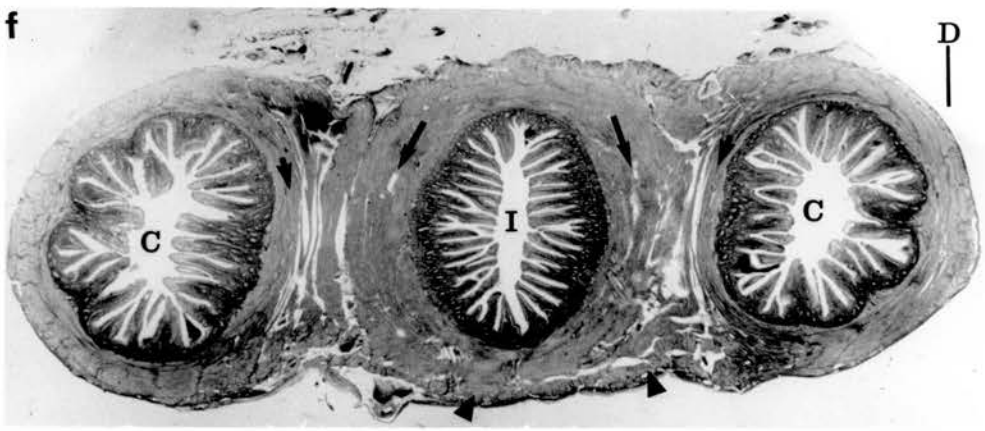
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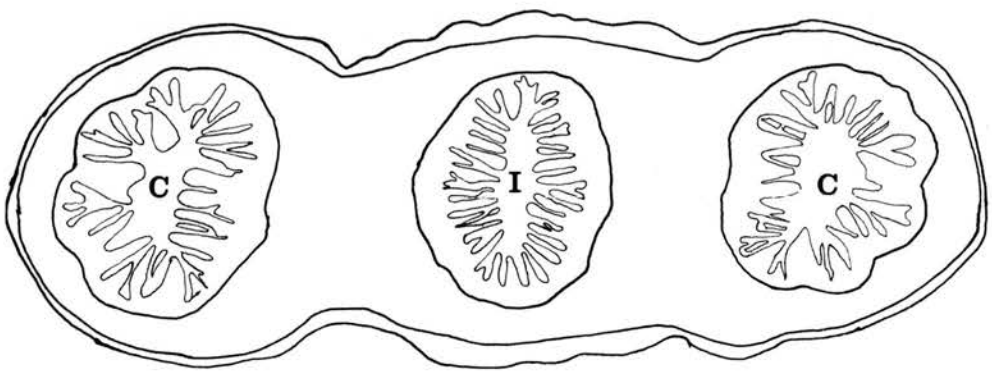
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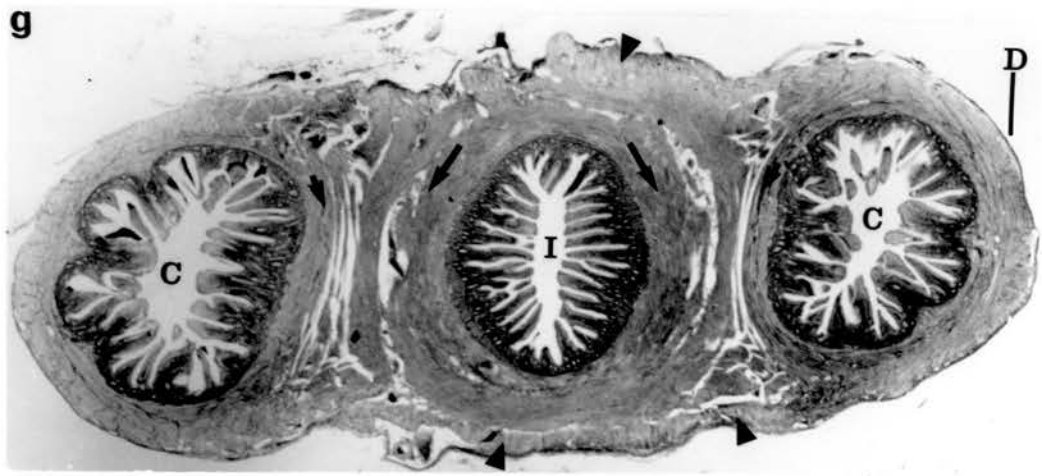
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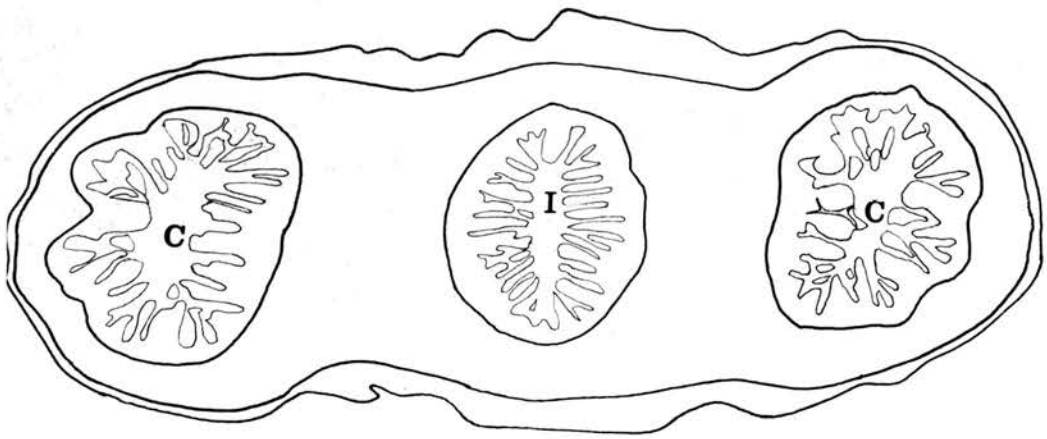
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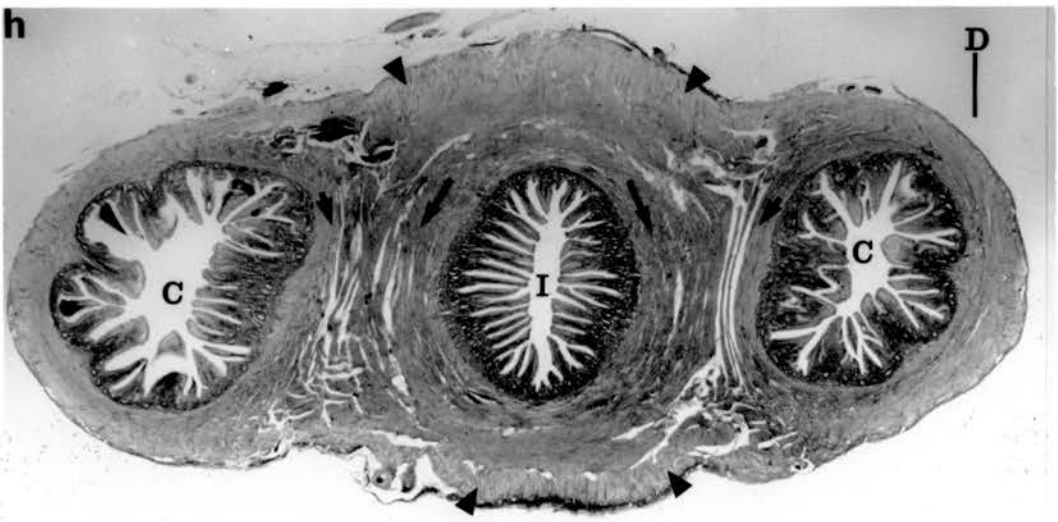
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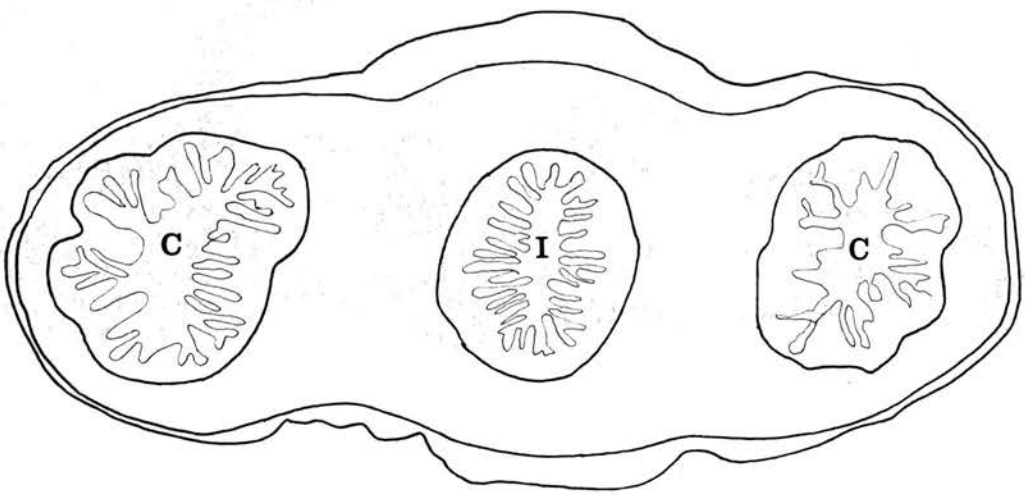
C
I
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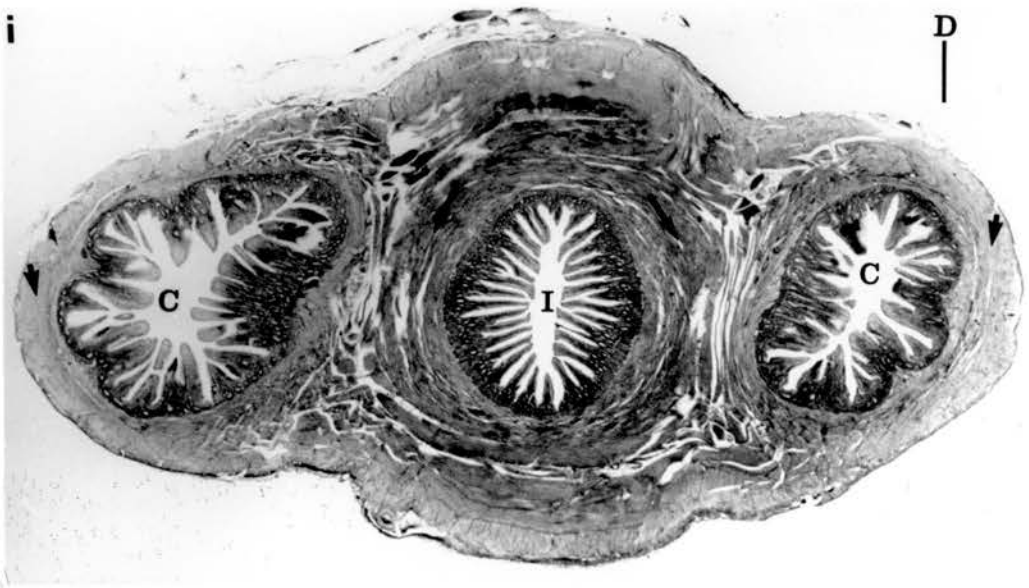
Fig. 9. Light micrographs of serial transverse sections of the ileo-caeco-rectal junction. The levels of the sections are shown in Fig. 8 . C, caecum; D, dorsal; I, ileum; IP, ileal papilla; R, rectum. Masson's trichrome stain. X 10.

i, j, k:

At these levels the circular muscle layer reaches its greatest thickness at the base of the ileal papilla and in the medial walls of the caecal orifice (long arrows). Note at this level the circular muscle layer in the lateral walls (short arrows) of the two caeca shows no increase in thickness. R, rectum.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D
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I
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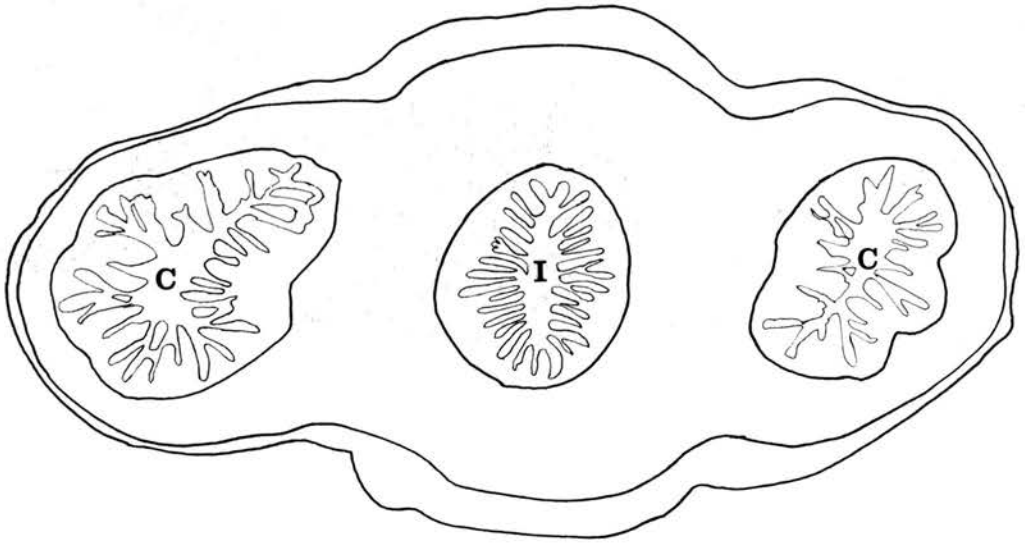
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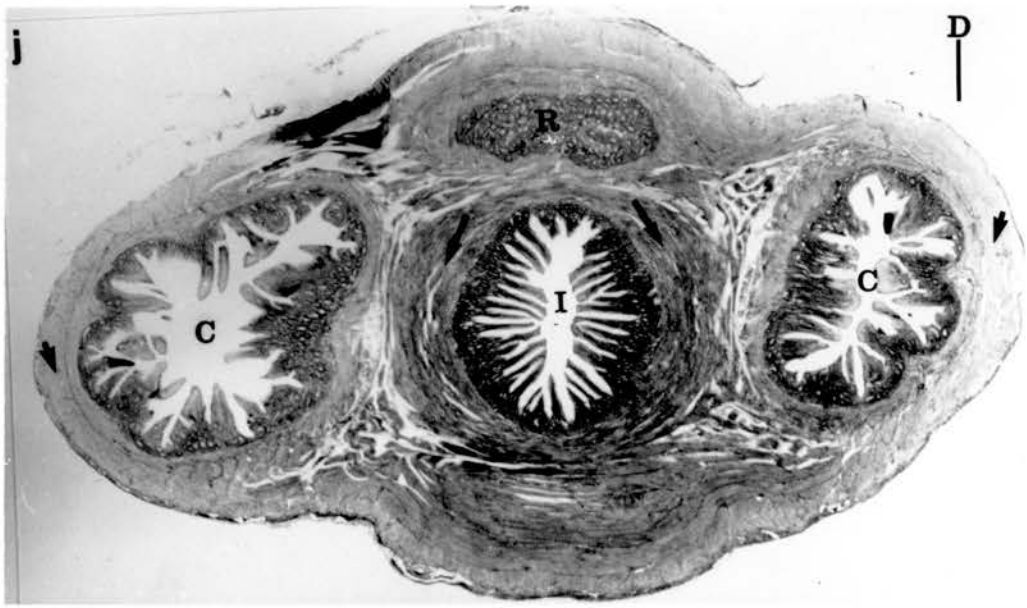
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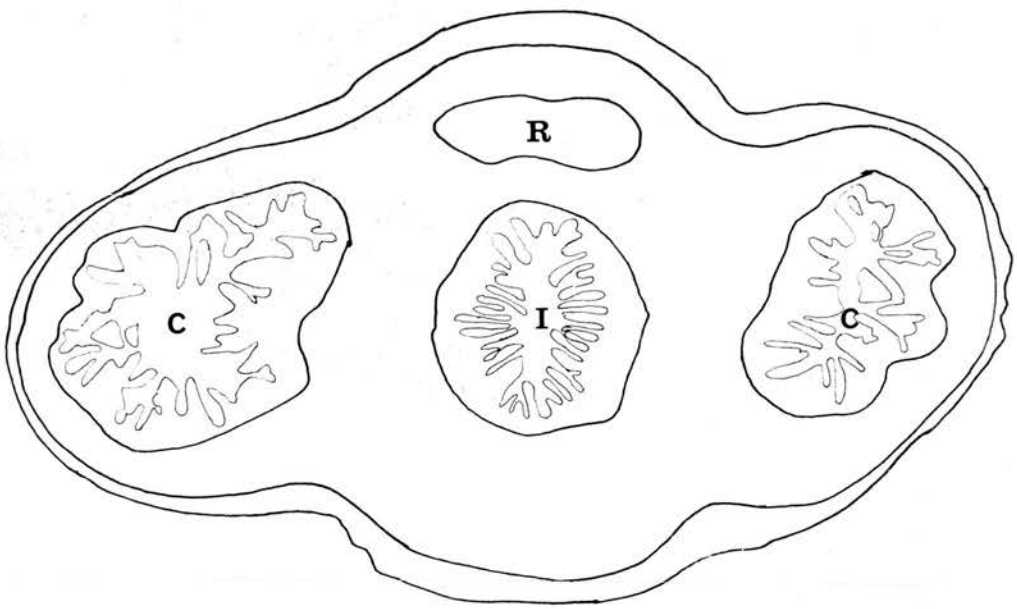
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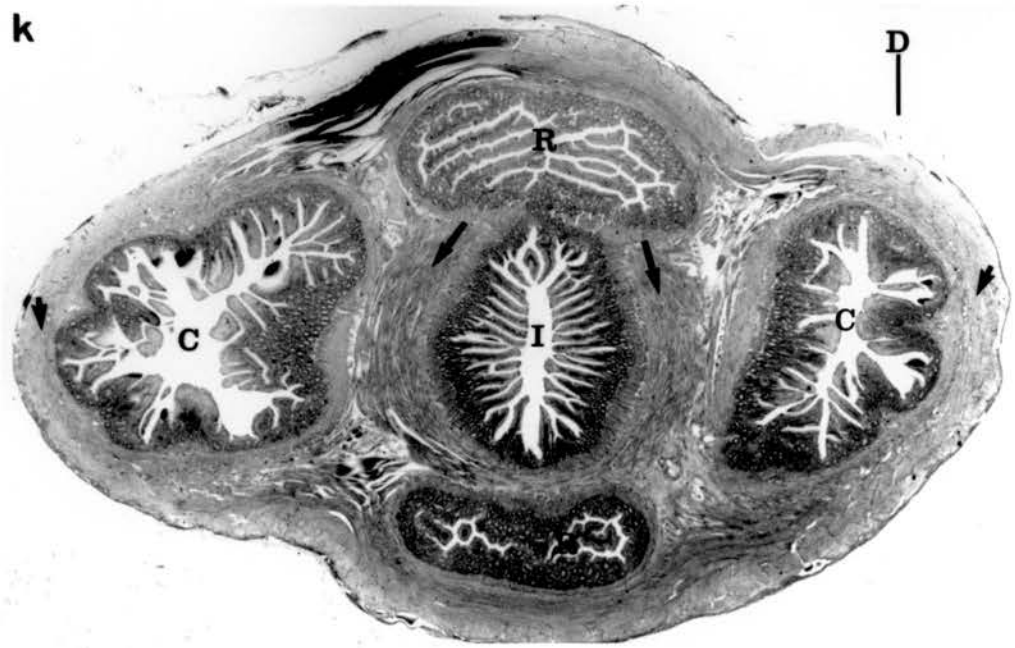
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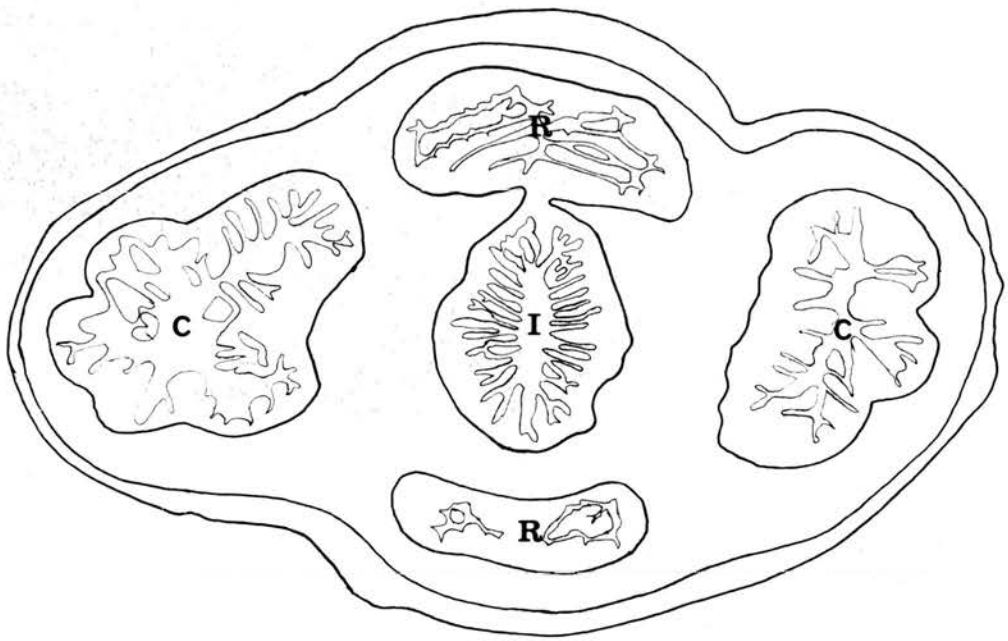
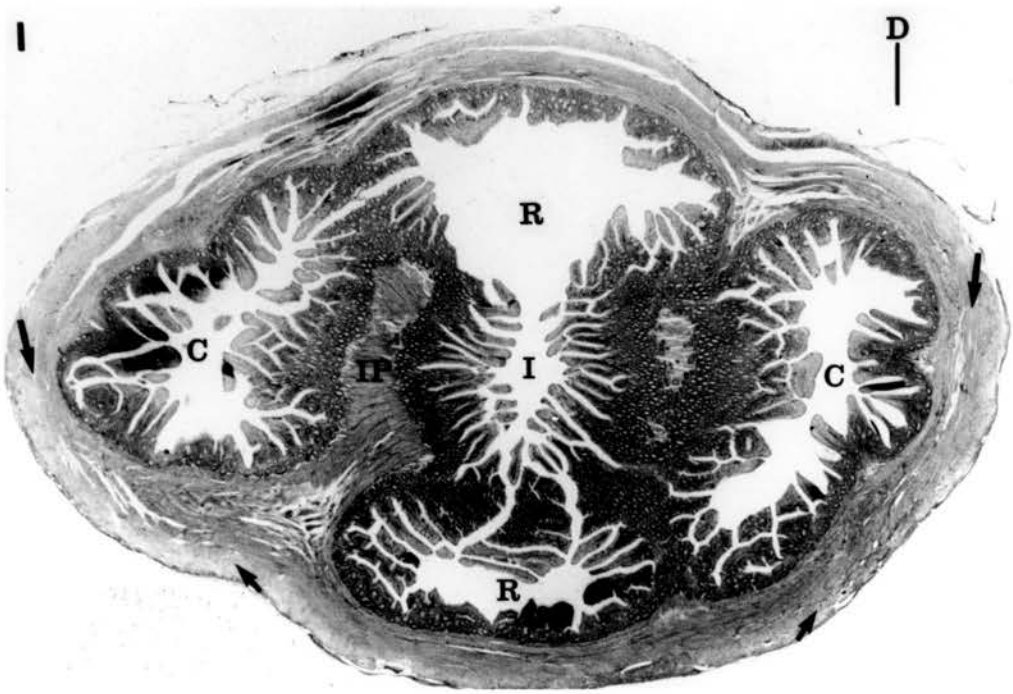

R
C
I
C
R

Fig. 9. Light micrographs of serial transverse sections of the ileo-caeco-rectal junction. The levels of the sections are shown in Fig. 8 . C, caecum; D, dorsal; I, ileum; IP, ileal papilla; R, rectum. Masson's trichrome stain. X 10.

l, m, n, o:

The ileal papilla (IP) appears in the centre of the rectum as unattached tissue. At this level the circular muscle layer in the lateral walls of the caecal orifices (long arrows) increases in thickness. The longitudinal muscle layer (short arrows) also shows a slight increase in thickness and is continuous with the longitudinal muscle layer of the rectum.


I
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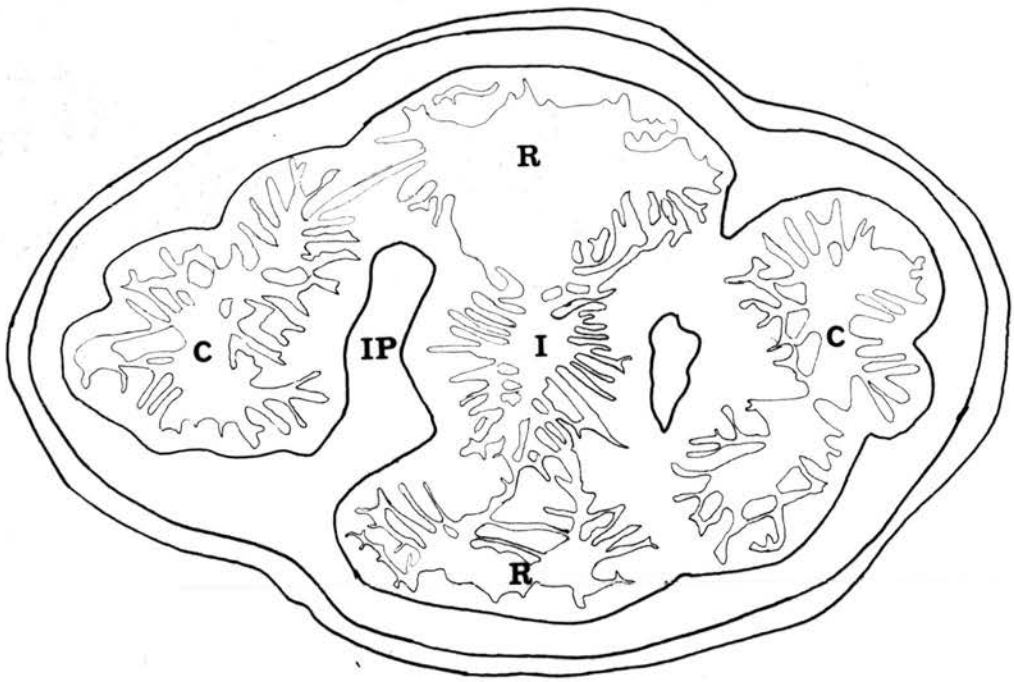
R
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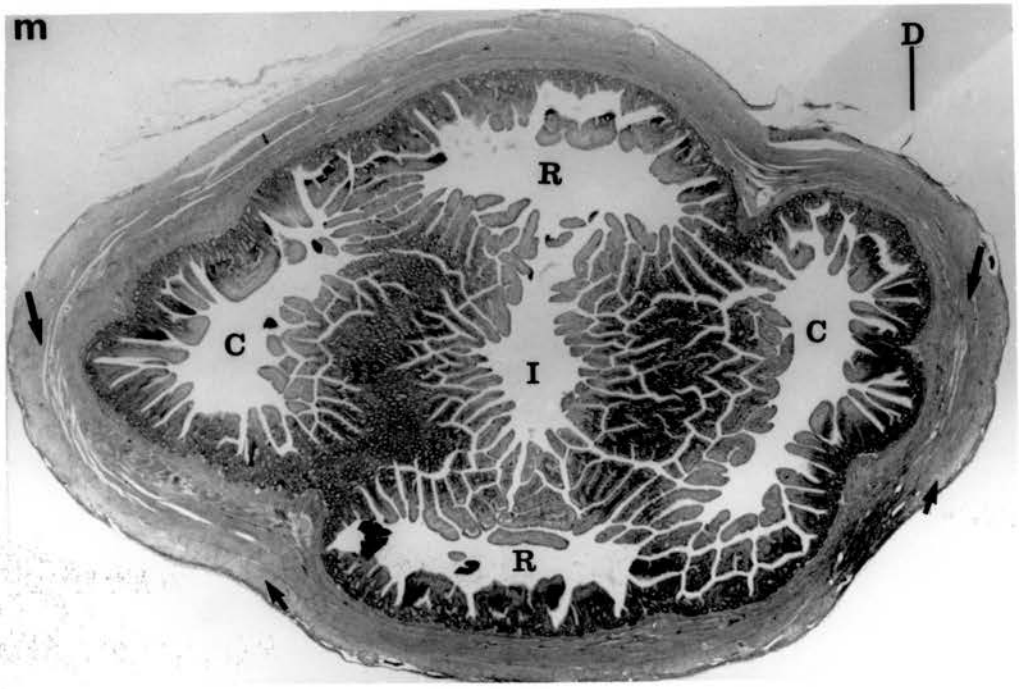
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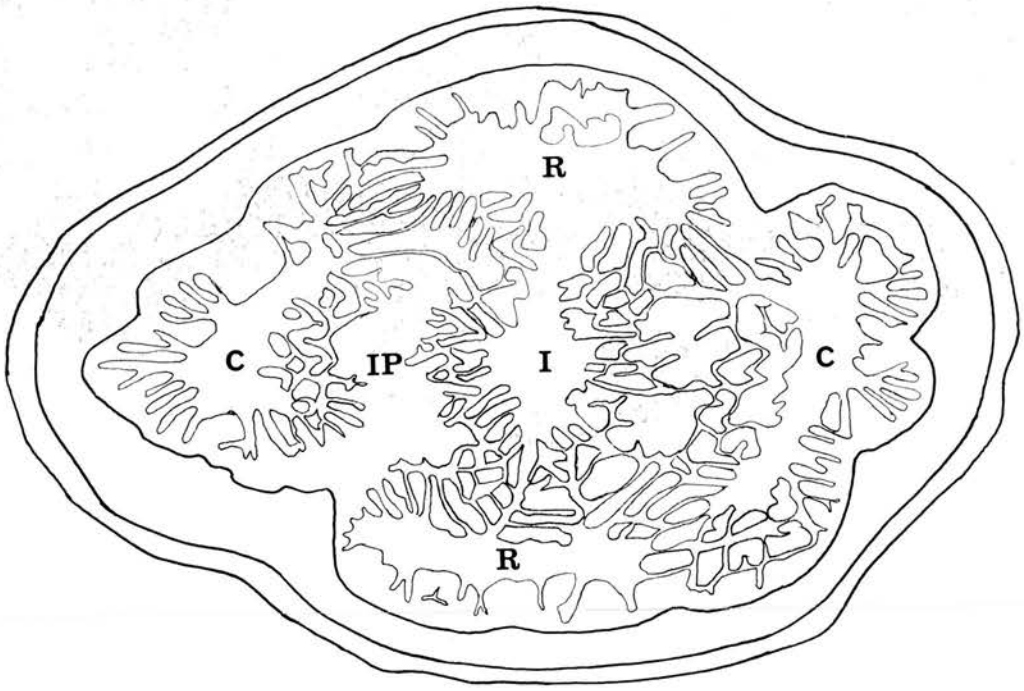
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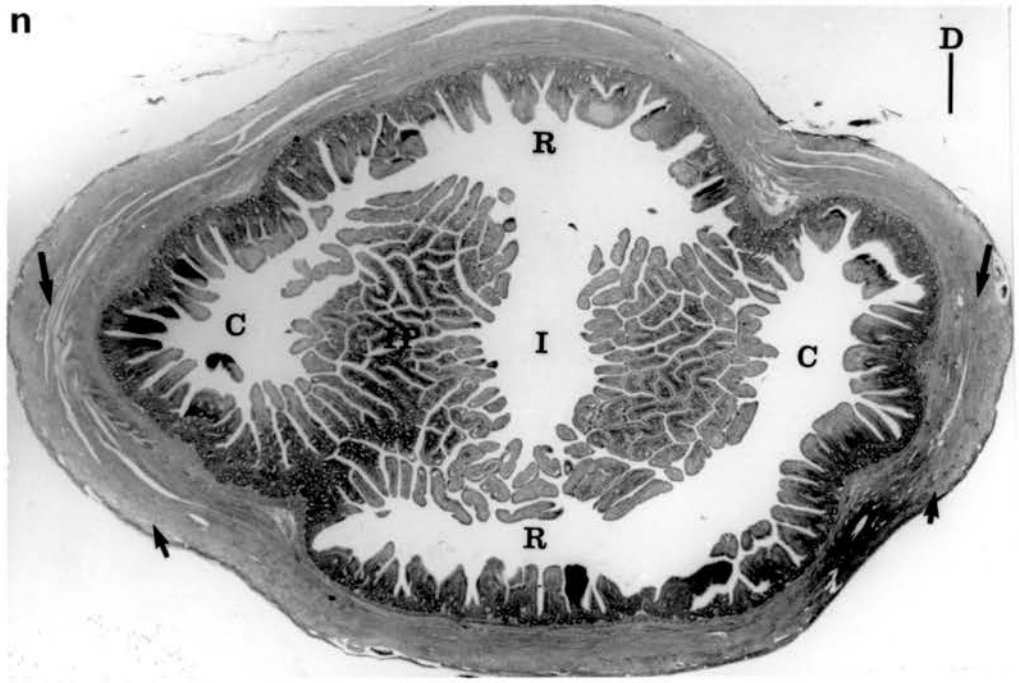

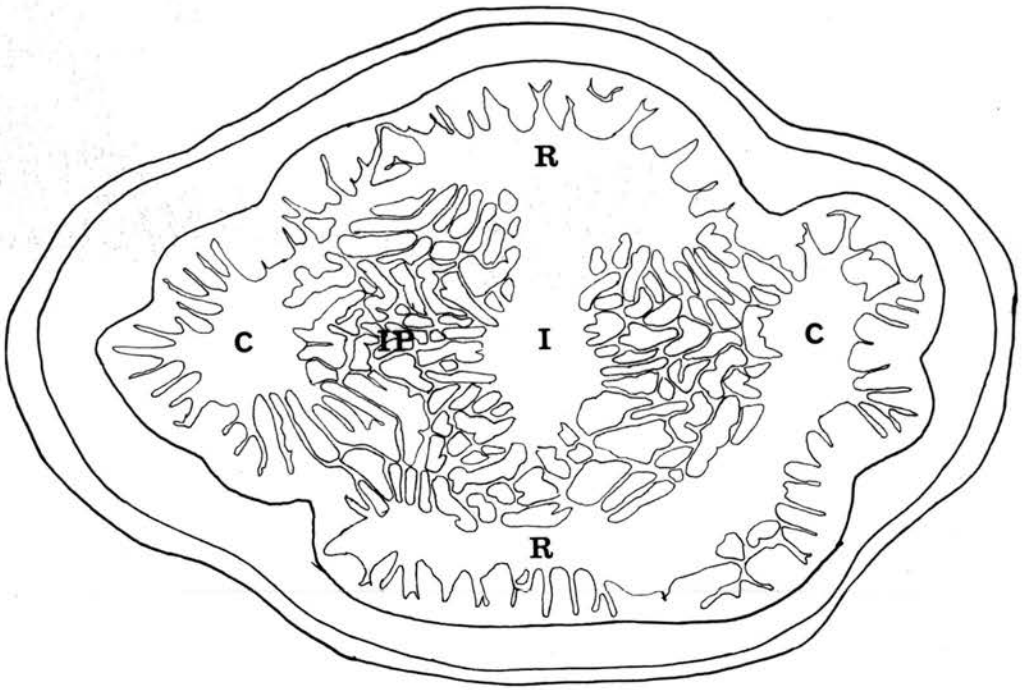

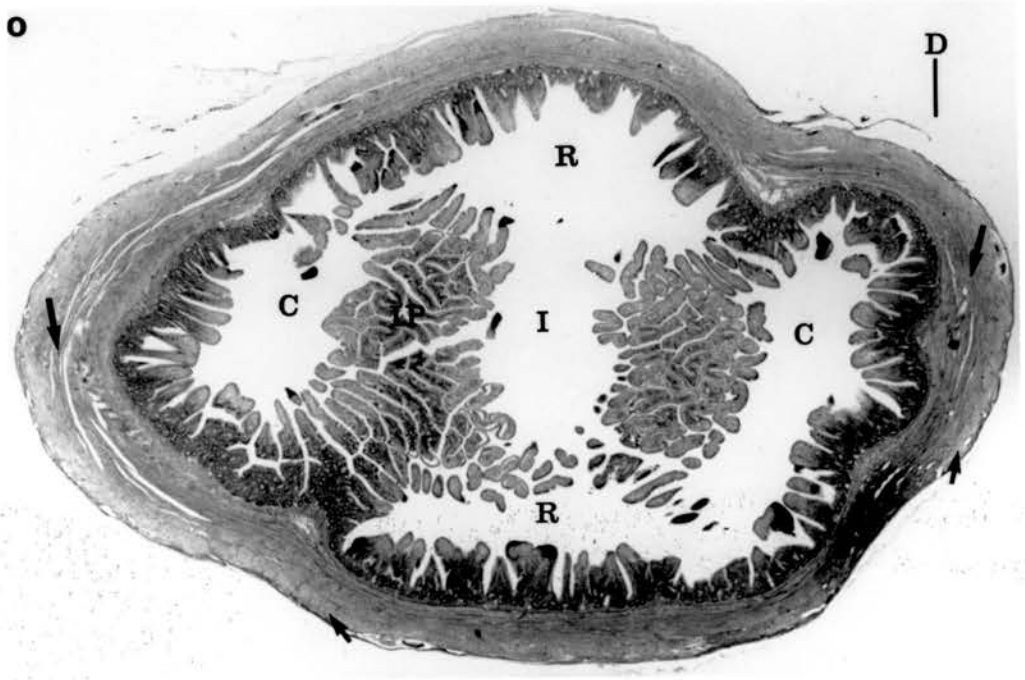
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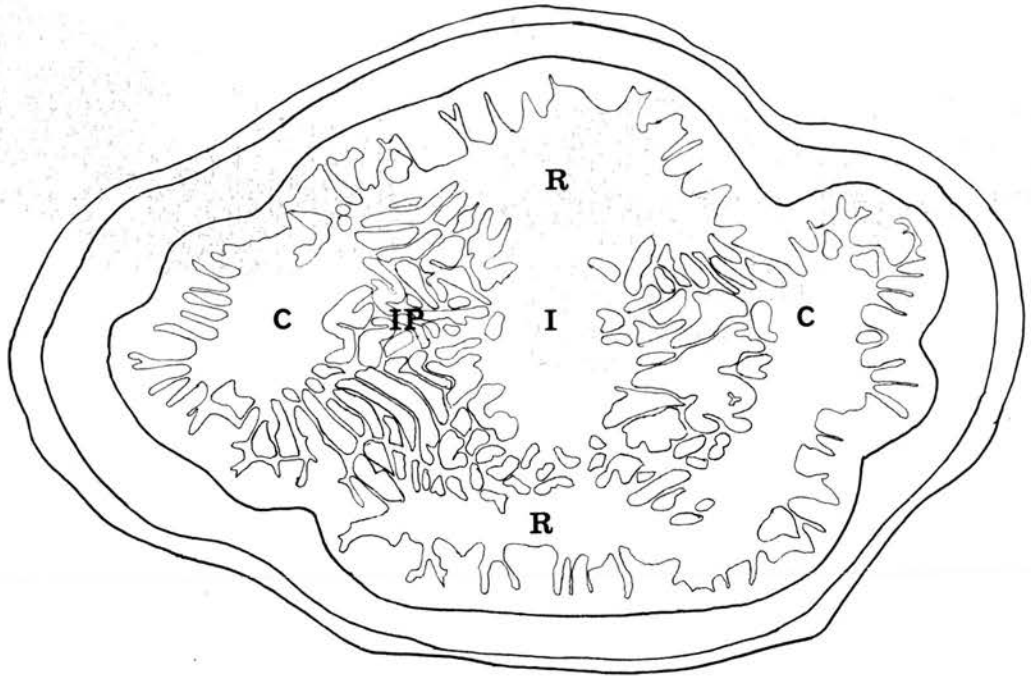
R
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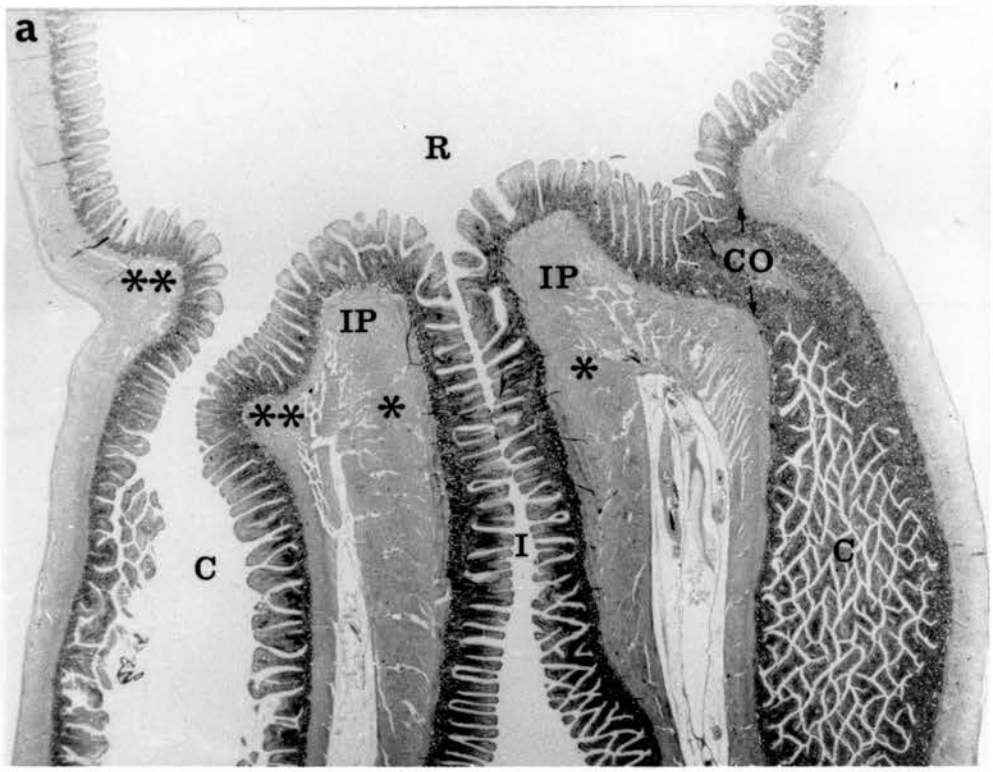
Fig. 10. Light micrographs of serial longitudinal sections of the ileo-caeco-rectal junction. C, caecum; CO, caecal orifice; I, ileum; IP, ileal papilla; R, rectum. X 10.

a, b, c, d:

The terminal part of the ileum has a funnel-shaped lumen which decreases in diameter towards the base of the ileal papilla and then slightly increases at the ileo-rectal orifice. The circular muscle layer at the base of the ileal papilla (*) and at the origins of the right and left caeca (**) is markedly increased in thickness. Note the thickened muscle at the base of the ileal papilla fuses on either side with the medial parts of the thickened muscle of the caecal orifices. The thickened muscle in the lateral parts of the caecal orifices are continuous with the circular muscle layer of the rectum.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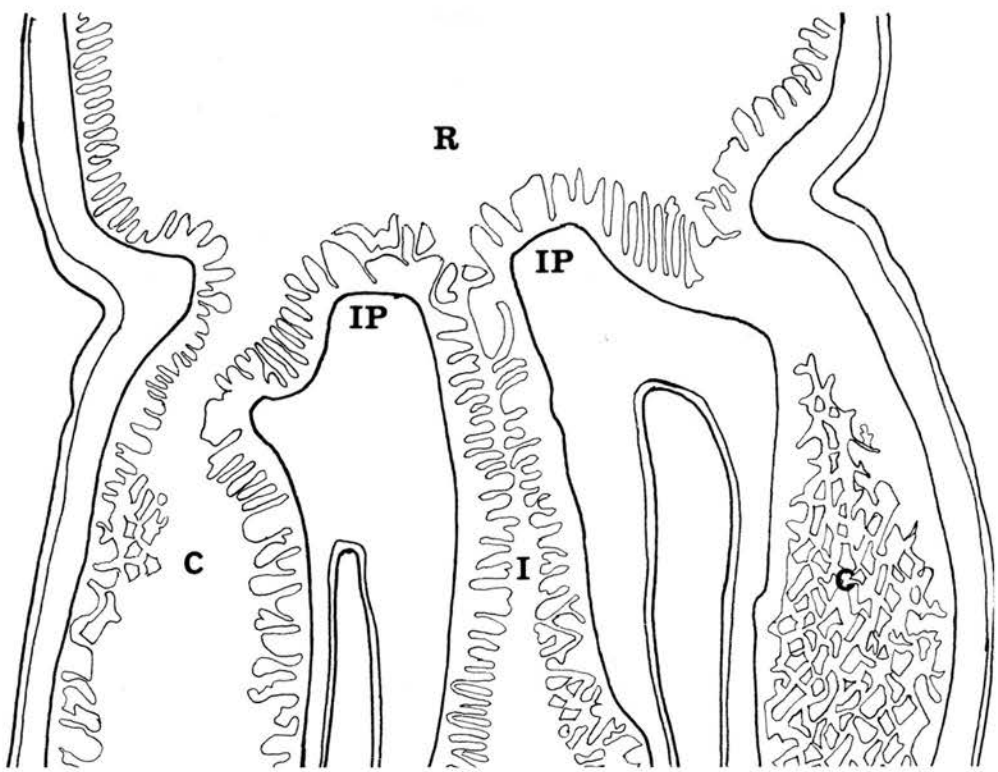
R
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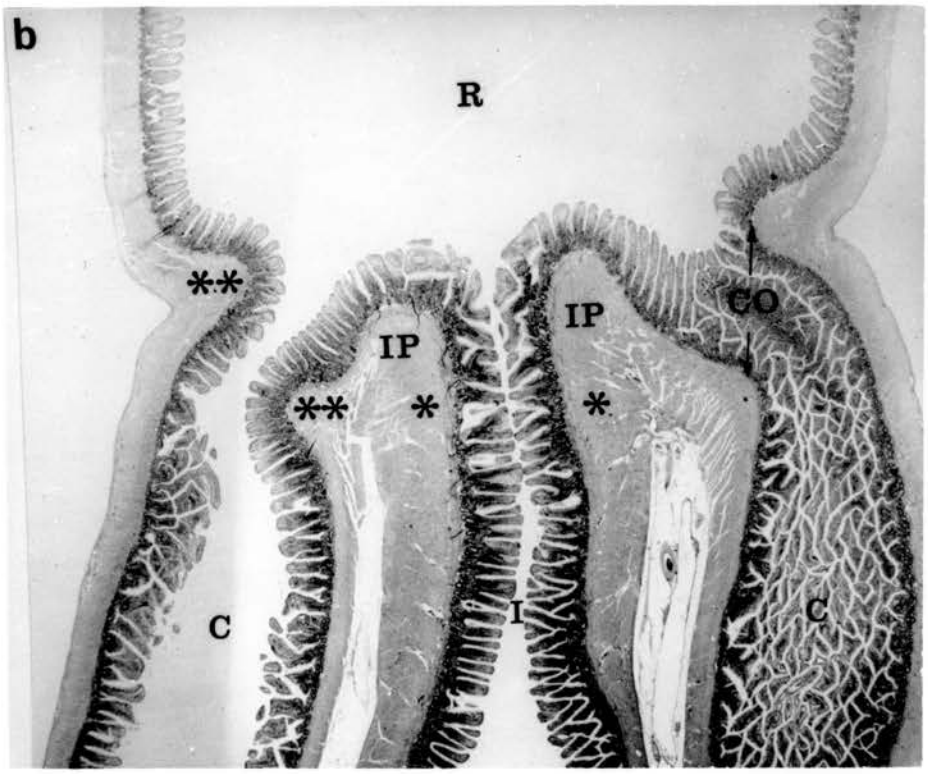
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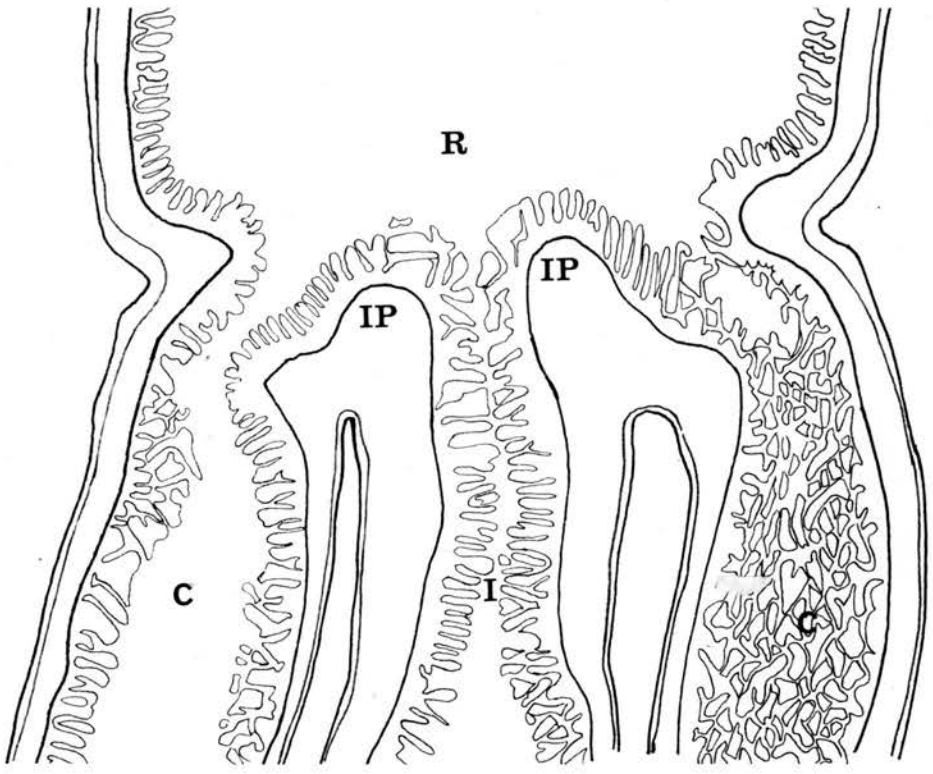
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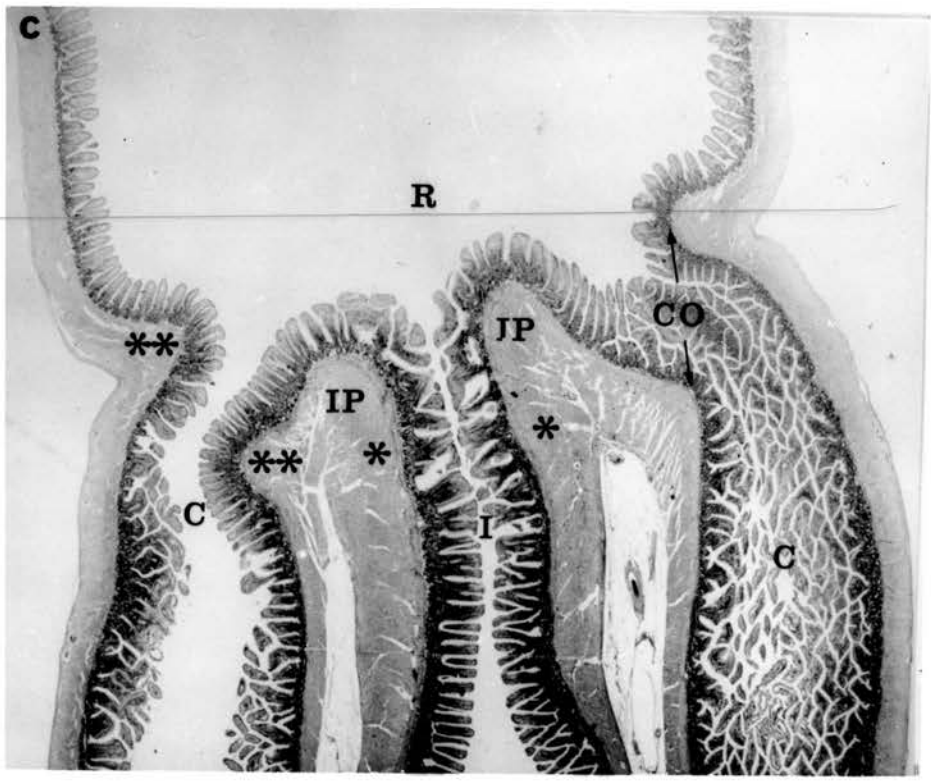
C
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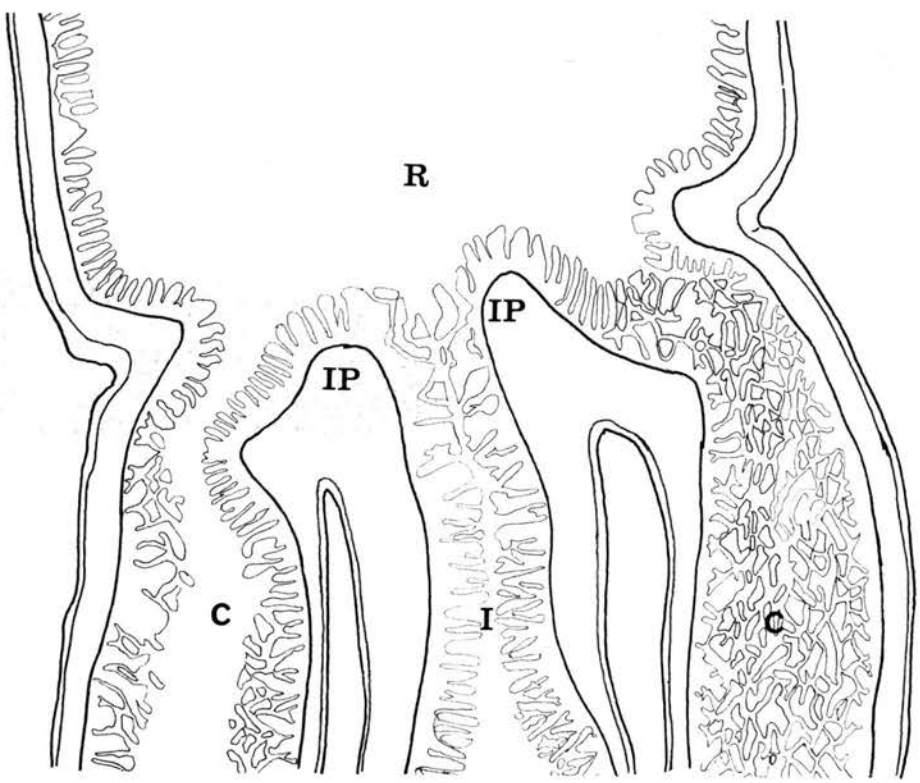
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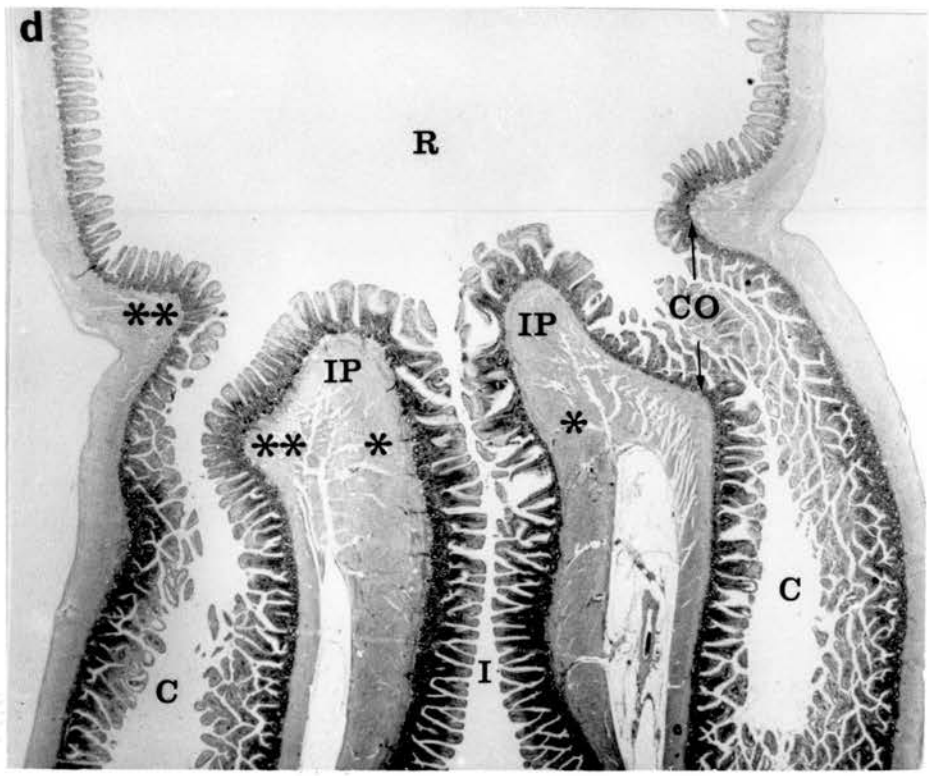
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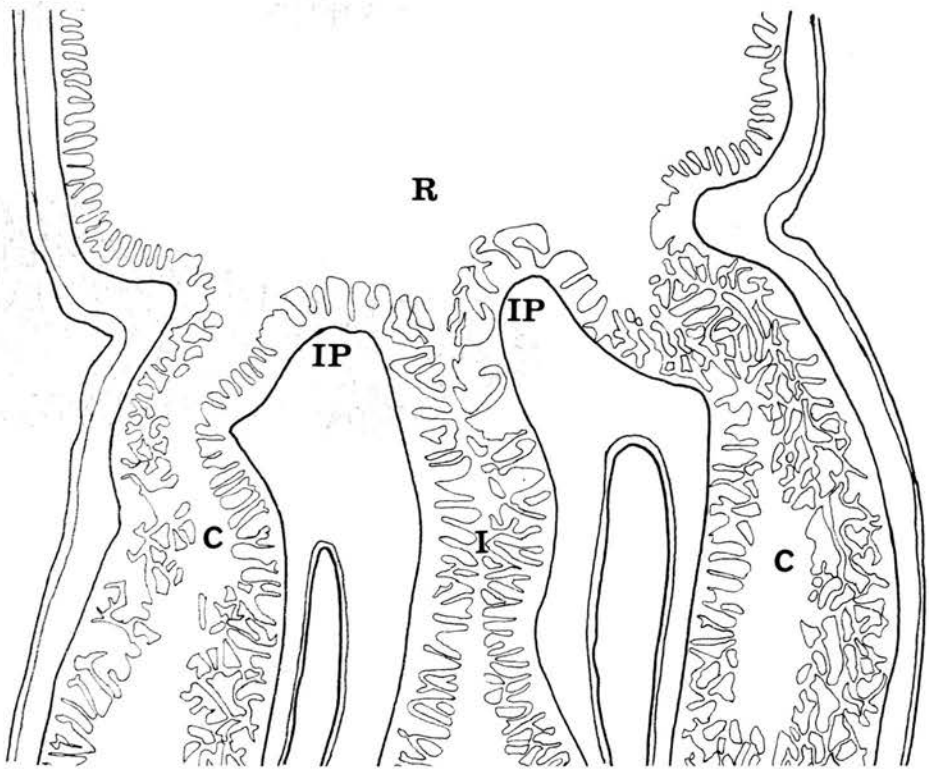
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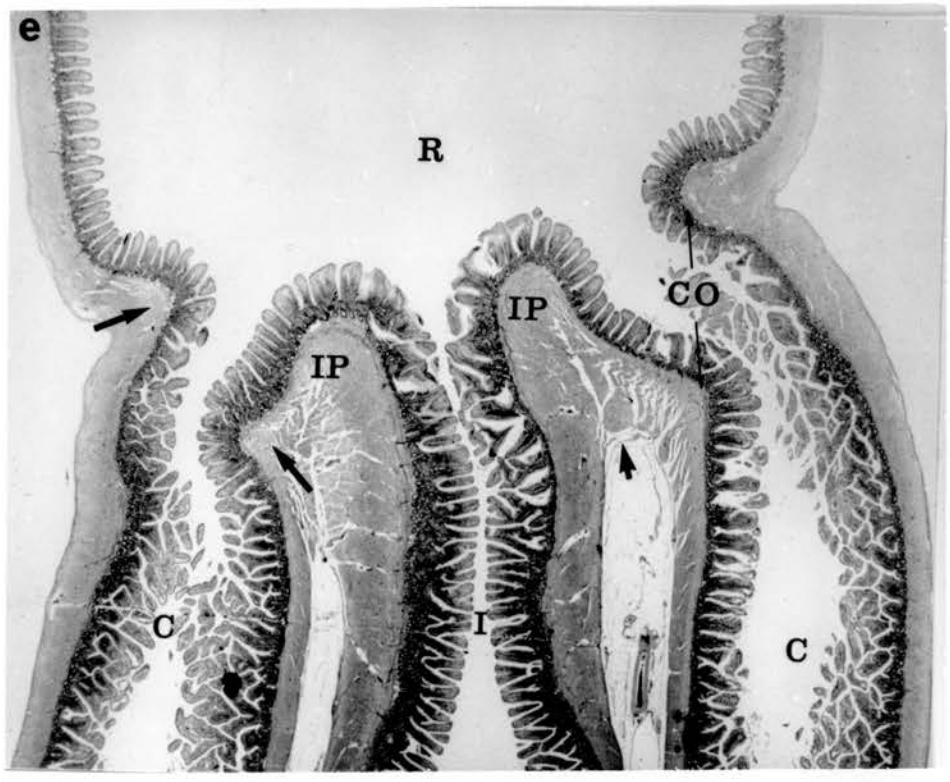
Fig. 10. Light micrographs of serial longitudinal sections of the ileo-caeco-rectal junction. C, caecum; CO, caecal orifice; I, ileum; IP, ileal papilla; R, rectum. X 10.

e, f, g:

The ileo-caeco-rectal junction is formed by a papilla-like protrusion of the ileum, the ileal papilla (IP), into the rectal lumen. The ventral part of the ileal papilla projects more caudally than the dorsal part. The thickened muscle around the caecal orifices (long arrows) protrudes into the lumen of the gut. The longitudinal muscle layer of the ileum (short arrow) does not extend into the ileal papilla. Immediately proximal to the base of the ileal papilla it becomes continuous on either side with the longitudinal muscle layer of the right and left caeca.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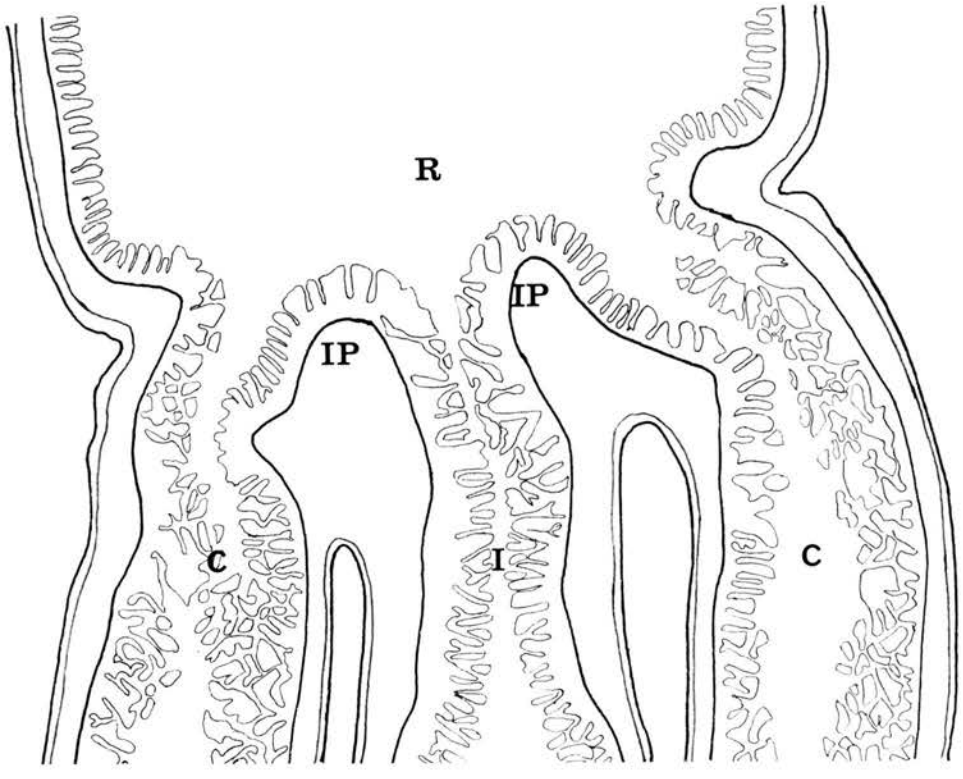
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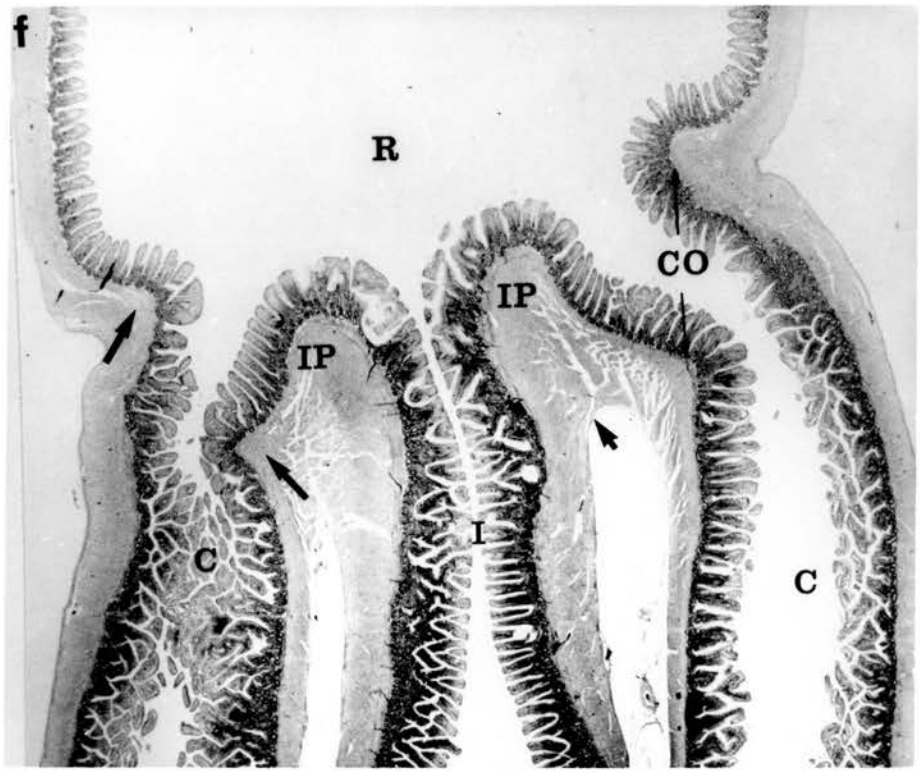
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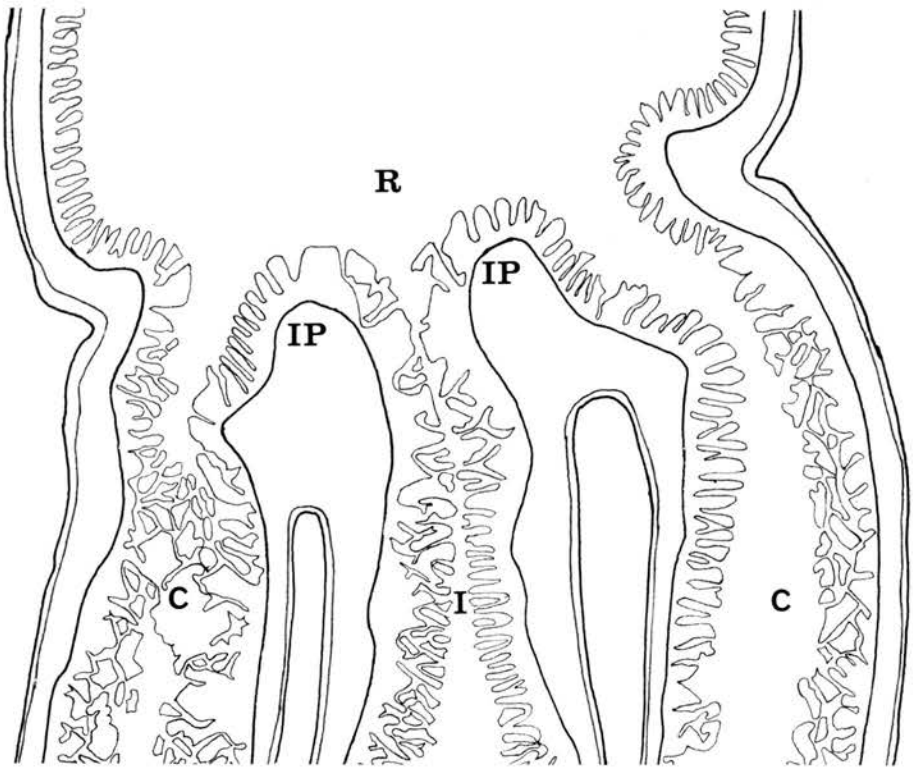
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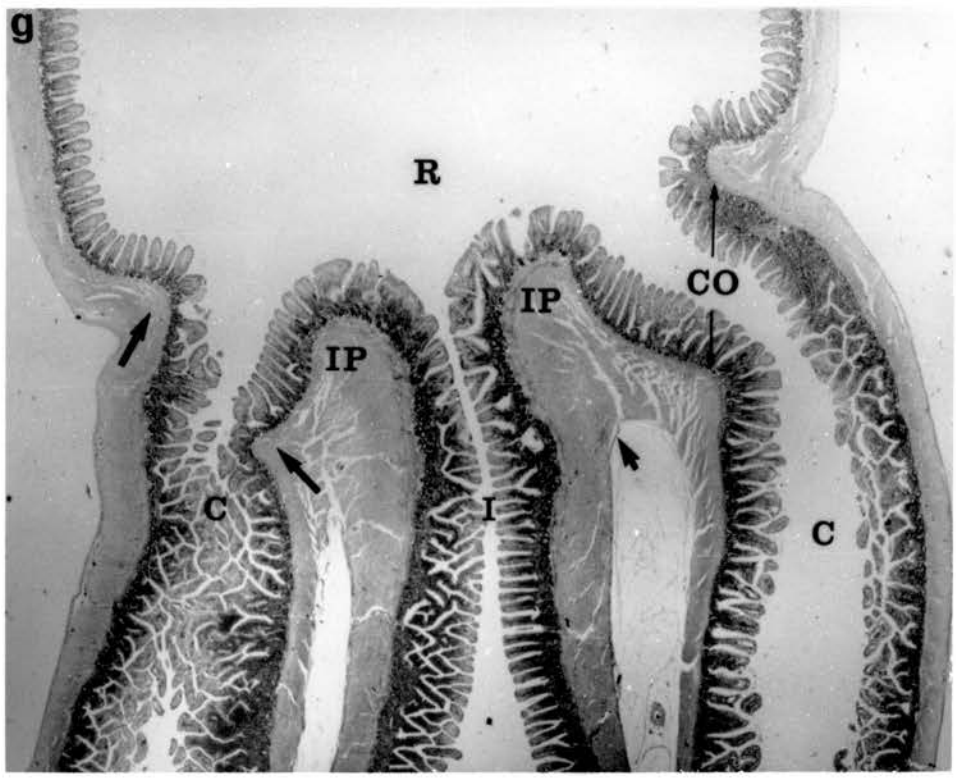
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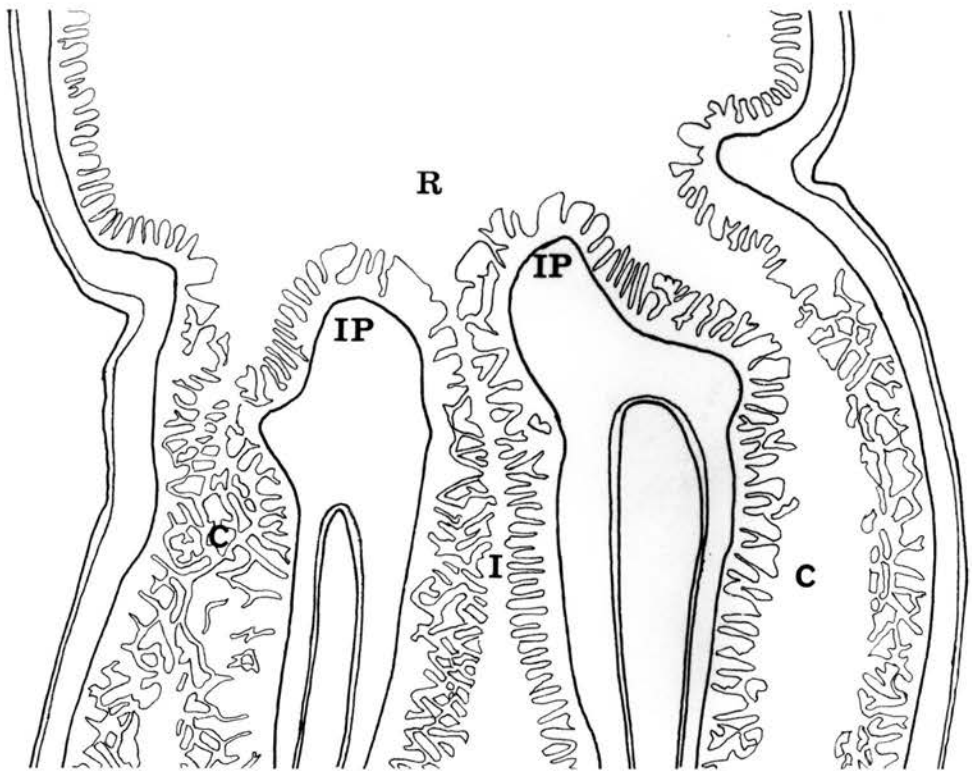
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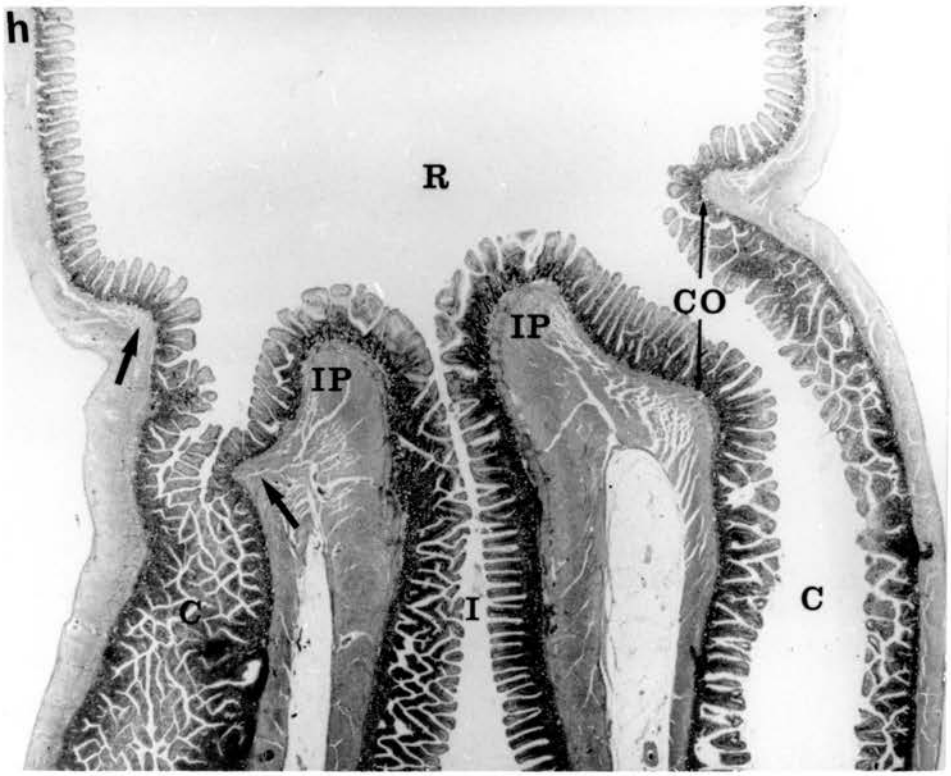
Fig. 10. Light micrographs of serial longitudinal sections of the ileo-caeco-rectal junction. C, caecum; CO, caecal orifice; I, ileum; IP, ileal papilla; R, rectum. X 10.

h, i, j:

The circular muscle layer of the ileum 5 mm from the ileo-caeco-rectal junction is increased gradually in thickness at the base of the ileal papilla (IP). At the apex of the papilla it becomes slightly thinner. As each caecum joins the gut obliquely the medial and lateral parts of the thickened muscle (long arrows) appear to lie at different levels. The longitudinal muscle layer at the origins of the caeca (short arrows) increases slightly in thickness. The lateral part becomes continuous caudally with the longitudinal muscle of the rectum and medially with the longitudinal muscle layer of the ileum.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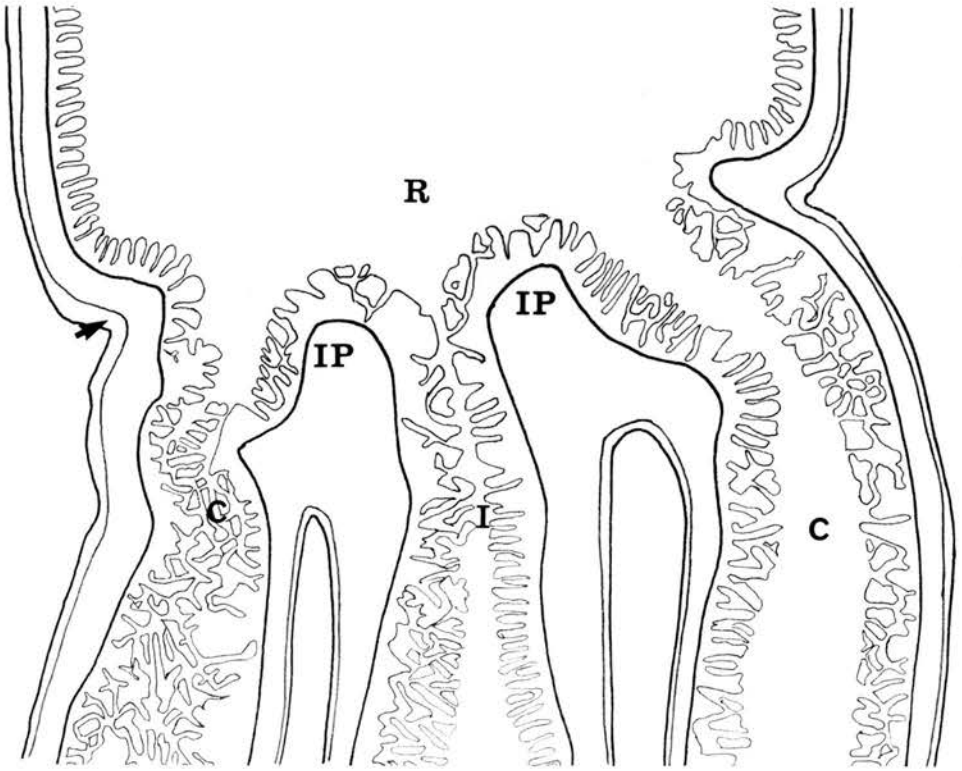
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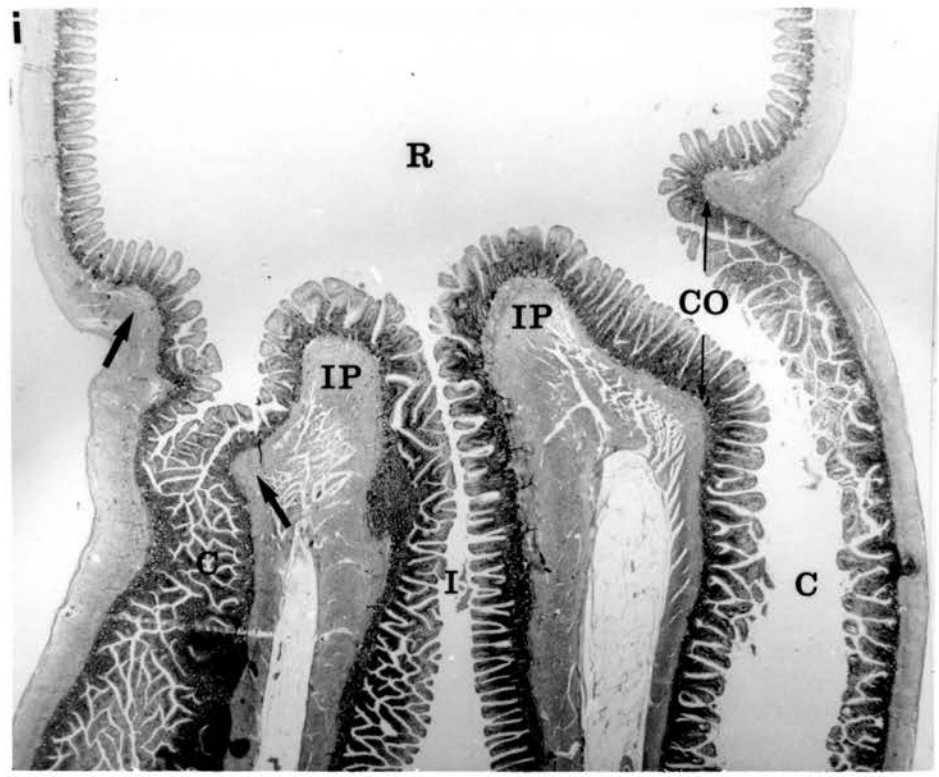
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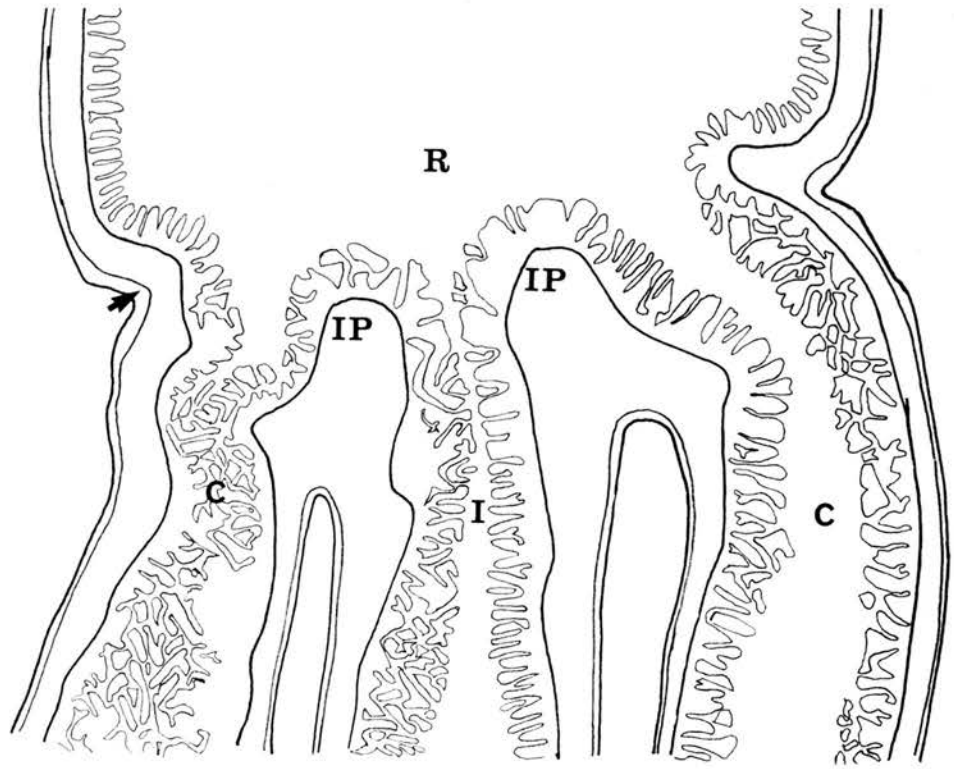
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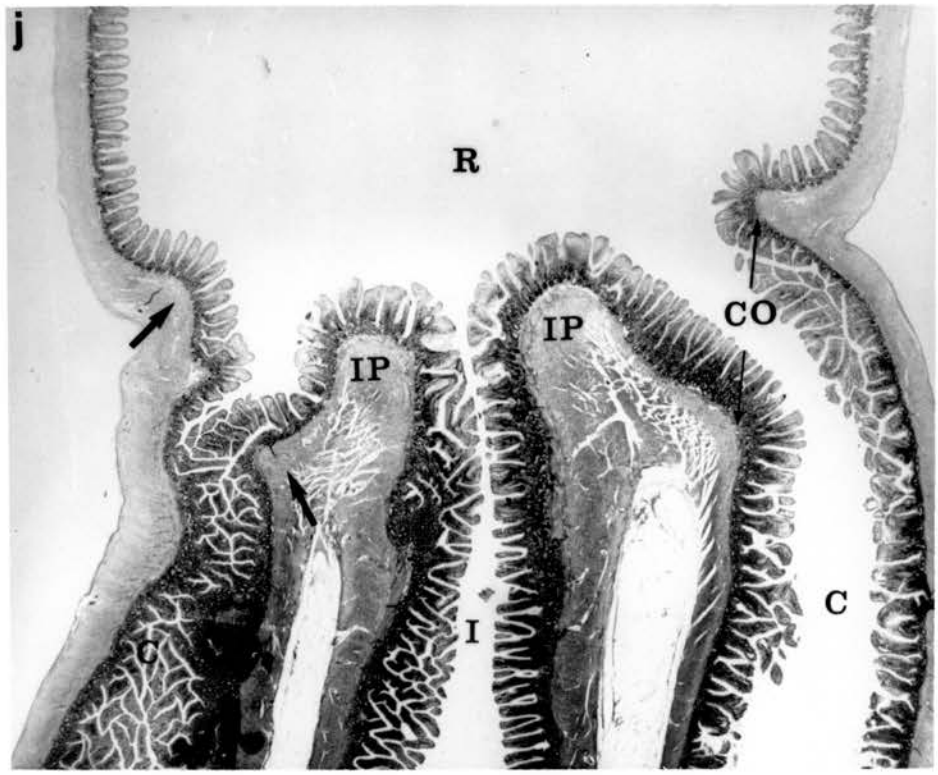
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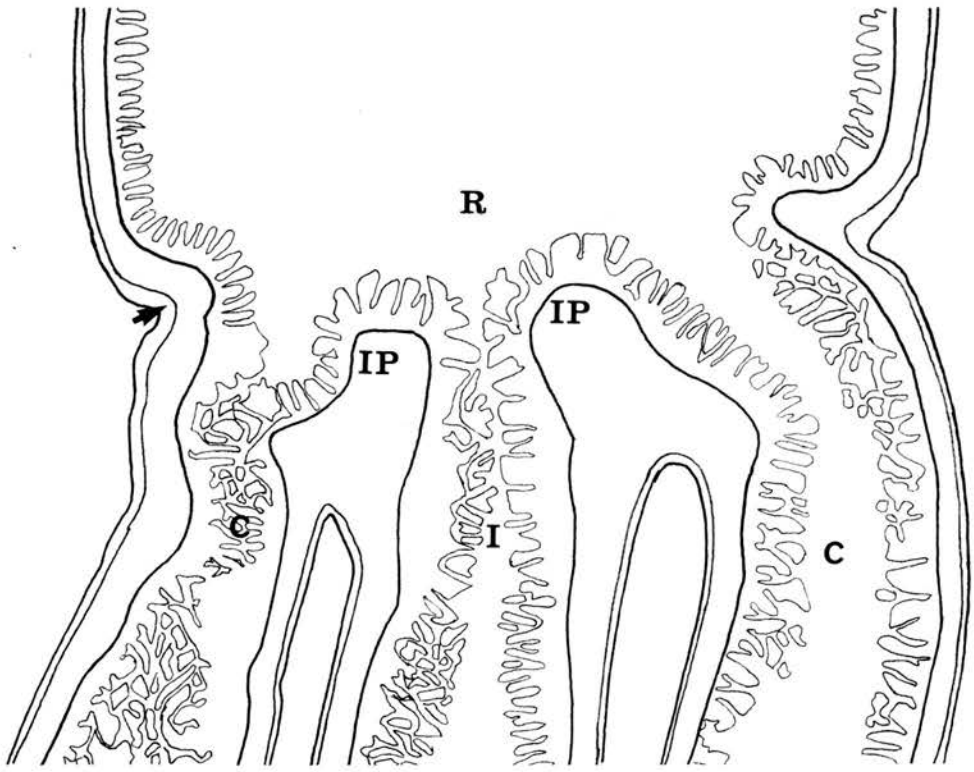
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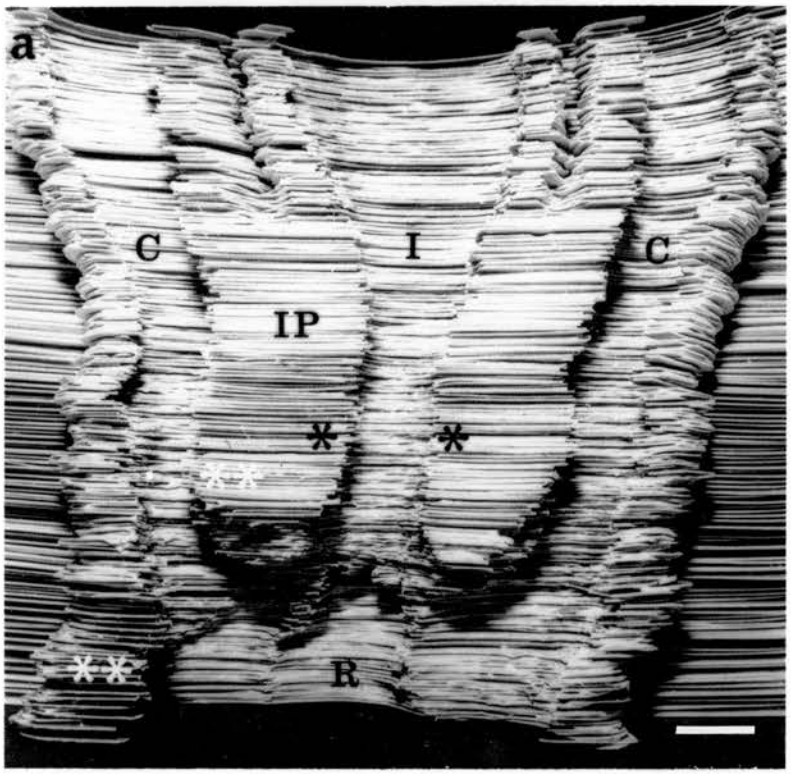
Fig. 11. 3-D photographic reconstruction models of the circular muscle layer at the ileo-caeco-rectal junction in two birds (a and b). At the ileal papilla (IP) the ileal circular muscle (*) thickens forming a muscular ring which fuses on either side with thickened caecal circular muscle (**). The scale bar refers to the width of the preparation and not the length. To exaggerate the thickenings of the muscle so that they can be seen more clearly the length magnification was reduced by a factor of X 0.5. C, caecum; I, ileum; R, rectum. Scale, 1 mm.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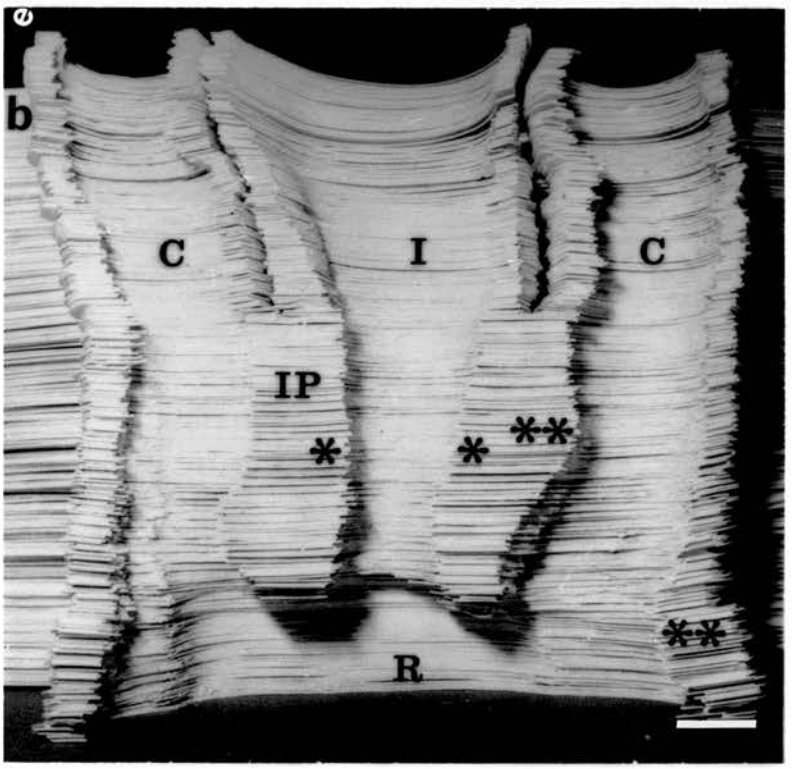
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(4) Ultrastructure of the Muscle Cells in the Region of the Ileo-Caeco-Rectal Junction.

(A) Scanning electron microscopic observations of the musculature.

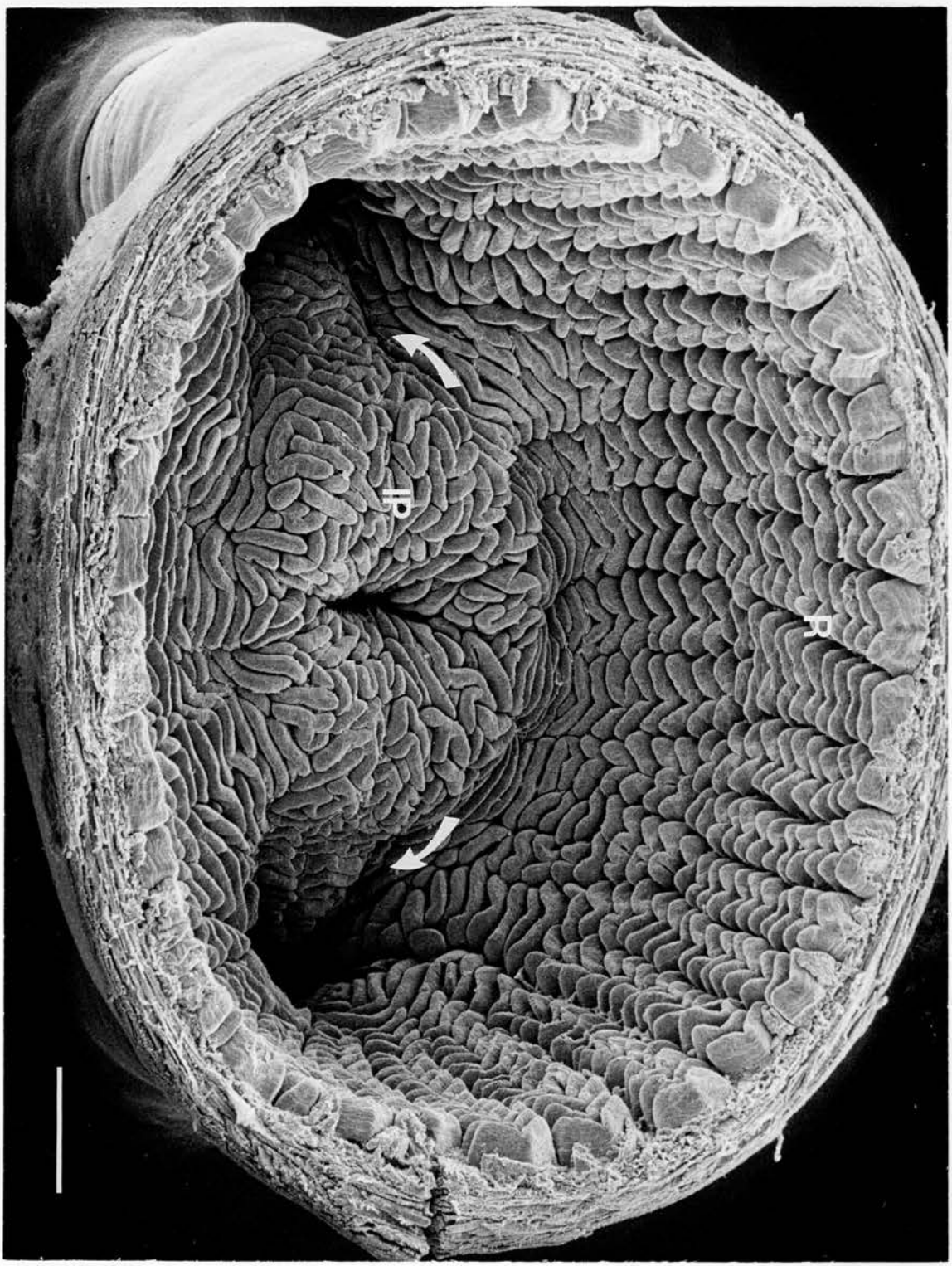
The ileo-caeco-rectal junction in the domestic duck was formed by a papilla-like protrusion of the ileum, the ileal papilla, into the lumen of the rectum, the openings of the large right and left caeca lying on either side of the papilla ventrolaterally (Fig. 12). At the base of the ileal papilla The circular muscle was markedly thickened to form a thick muscular ring (Fig. 13). At the base of each caecum the circular muscle was also thickened forming a ring around the caecal orifice (Fig. 13). The three muscular rings, two around the orifices of the right and left caeca and one at the base of the ileal papilla, were continuous (Fig. 14).

(B) Transmission electron microscopic observations of the muscle cells.

(a) Muscle layers.

The muscle layers in the distended wall of the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction consisted of four closely apposed layers including the muscularis mucosae (inner longitudinal layer), the inner circular

Fig. 12. Scanning electron micrograph of the ileo-caeco-rectal junction. The junction is formed by the ileal papilla (IP) which protrudes caudally into the rectum (R), the openings of the right and left caeca (arrows) into the rectum lying lateral to the papilla. Scale, 1 mm.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R

Fig. 13. Montage of scanning electron micrographs of the ileo-caeco-rectal junction cut in longitudinal section. At the base of the ileal papilla the ileal circular muscle (*) is markedly thickened forming a thick muscular ring. At the base of each caecum the circular muscle (**) is also thickened forming a ring around the caecal orifice. C, caecum; I, ileum; R, rectum. Scale, 1 mm.

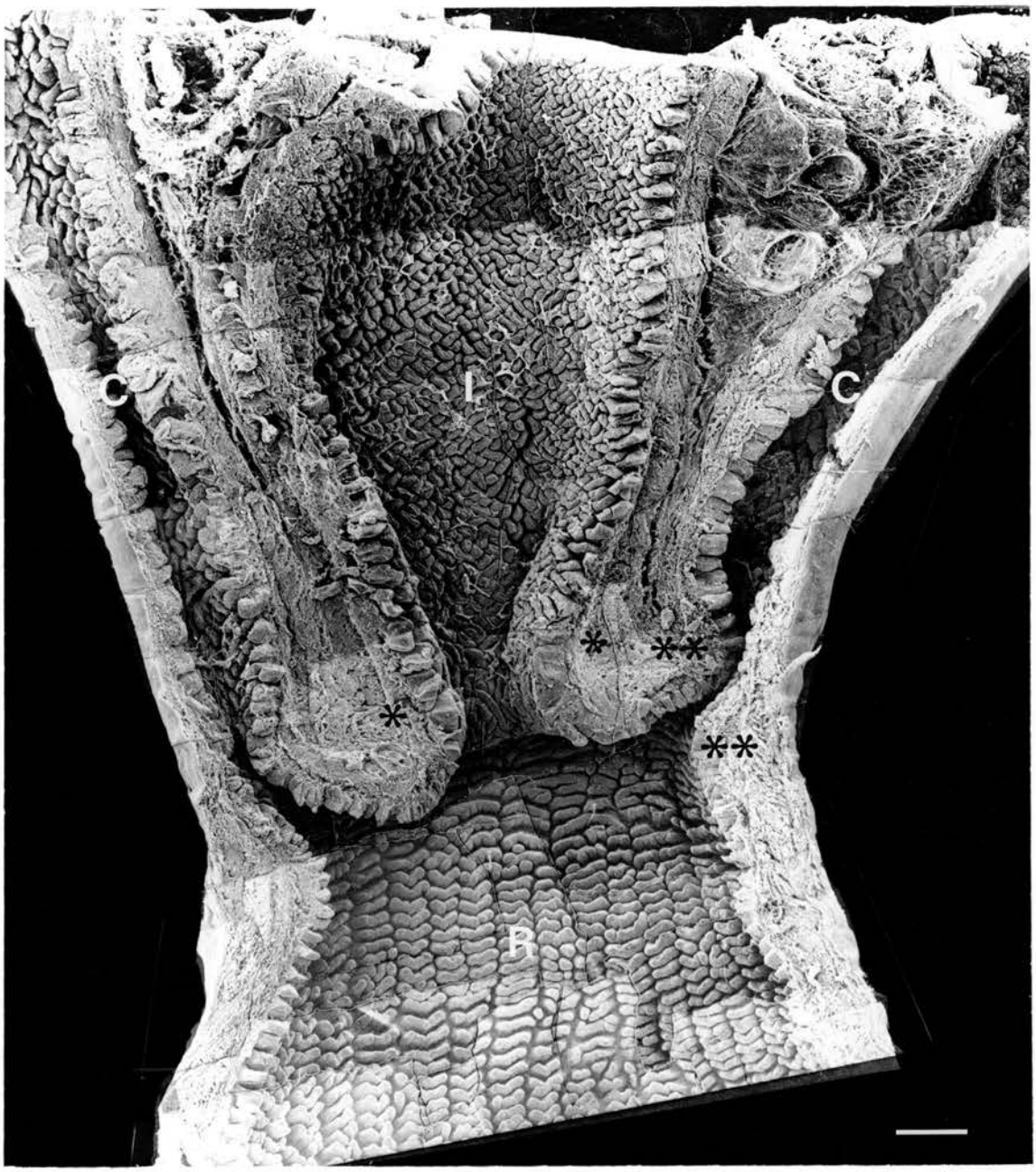
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Fig. 14. Scanning electron micrograph of the ileo-caeco-rectal junction. The three muscular rings, one around the base of the ileal papilla (large arrow) and two around the base of the right and left caeca (small arrows), are continuous. C, caecum; I, ileum. Scale, 1 mm.

C
C

layer, the outer circular layer, and the outer longitudinal layer.

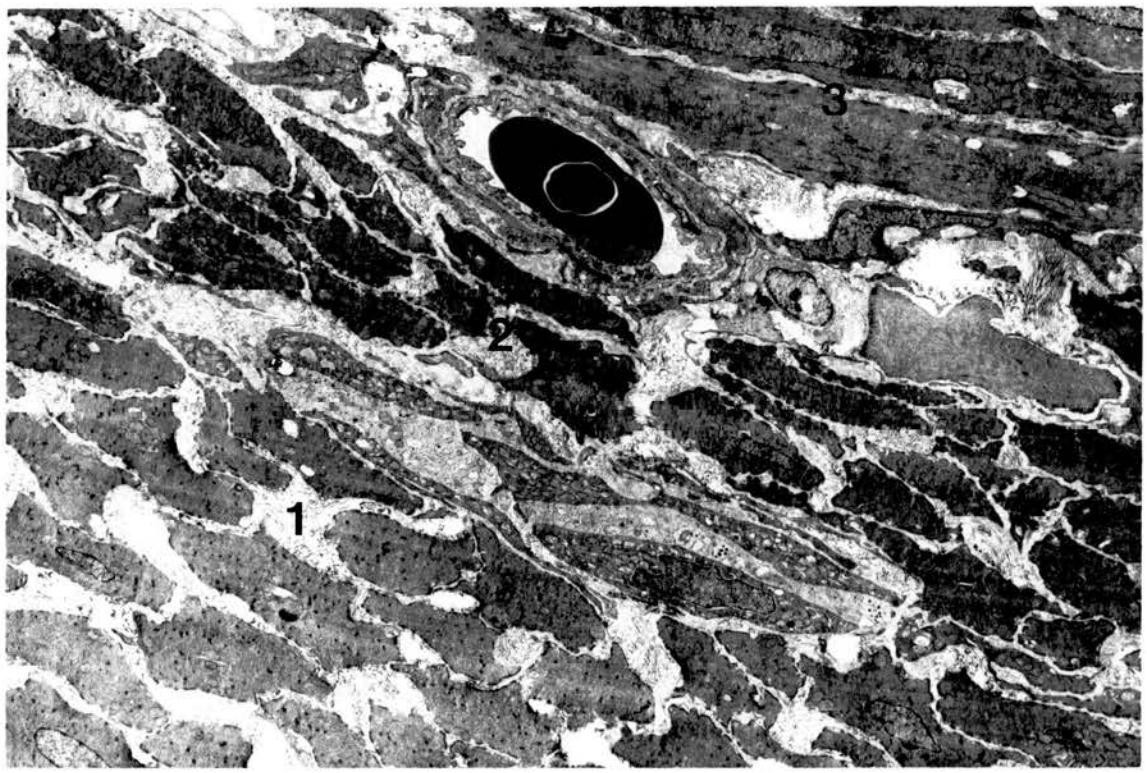
The muscularis mucosae (inner longitudinal layer) was 6-8 muscle cells thick and was separated from the circular muscle layer by a very thin sheet of connective tissue which increased in thickness when ganglia and blood vessels were present (Fig. 15). This connective tissue layer, however, was absent at many points and the muscle cells of the inner longitudinal layer came to be close to the muscle cells of the circular layer, the intercellular gap being reduced to about 50 nm.

The inner circular muscle layer was thick, and was clearly divided into an inner thin portion and an outer thick portion. The inner portion consisted of 2-6 rows of muscle cells. The cells were small and electron-dense and their long axis lay parallel to the long axis of the remaining cells of the circular layer (Fig. 15). They were separated from the outer portion of the circular layer by a wide layer of connective tissue containing many large nerve bundles and interstitial cells. The outer circular portion of the circular layer formed the bulk of the circular muscle. The muscle cells were larger and less electron-dense than those of the inner portion (Fig. 15).

The outermost layer of the muscle was the outer longitudinal layer which was 8-10 cells thick and was separated from the outer portion of the circular layer by a very thin sheet of connective tissue (Fig. 16). The cells of this layer at many points were directly apposed to the cells of the circular layer, the

Fig. 15. Transmission electron micrograph of the circular muscle of the ileum 5 mm from the ileo-caeco-rectal junction. The layer is divided into a thick outer relatively electron-lucent portion (1) and a thin inner relatively electron-dense portion (2). 3, muscularis mucosae. X 5000.

Fig. 16. Transmission electron micrograph of the circular muscle layer of the ileum 5 mm from the ileo-caeco-rectal junction. The outer circular portion (1) is separated from the outer longitudinal muscle layer (2) by a thin layer of connective tissue (ct). X 7500.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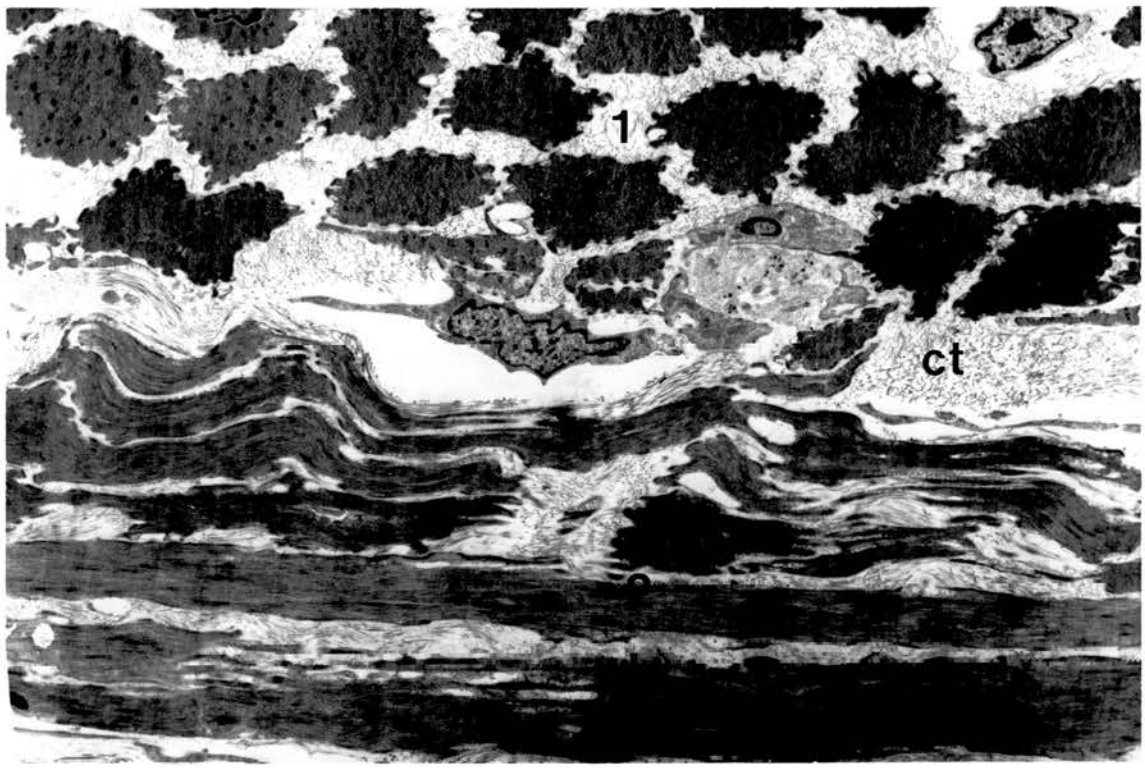
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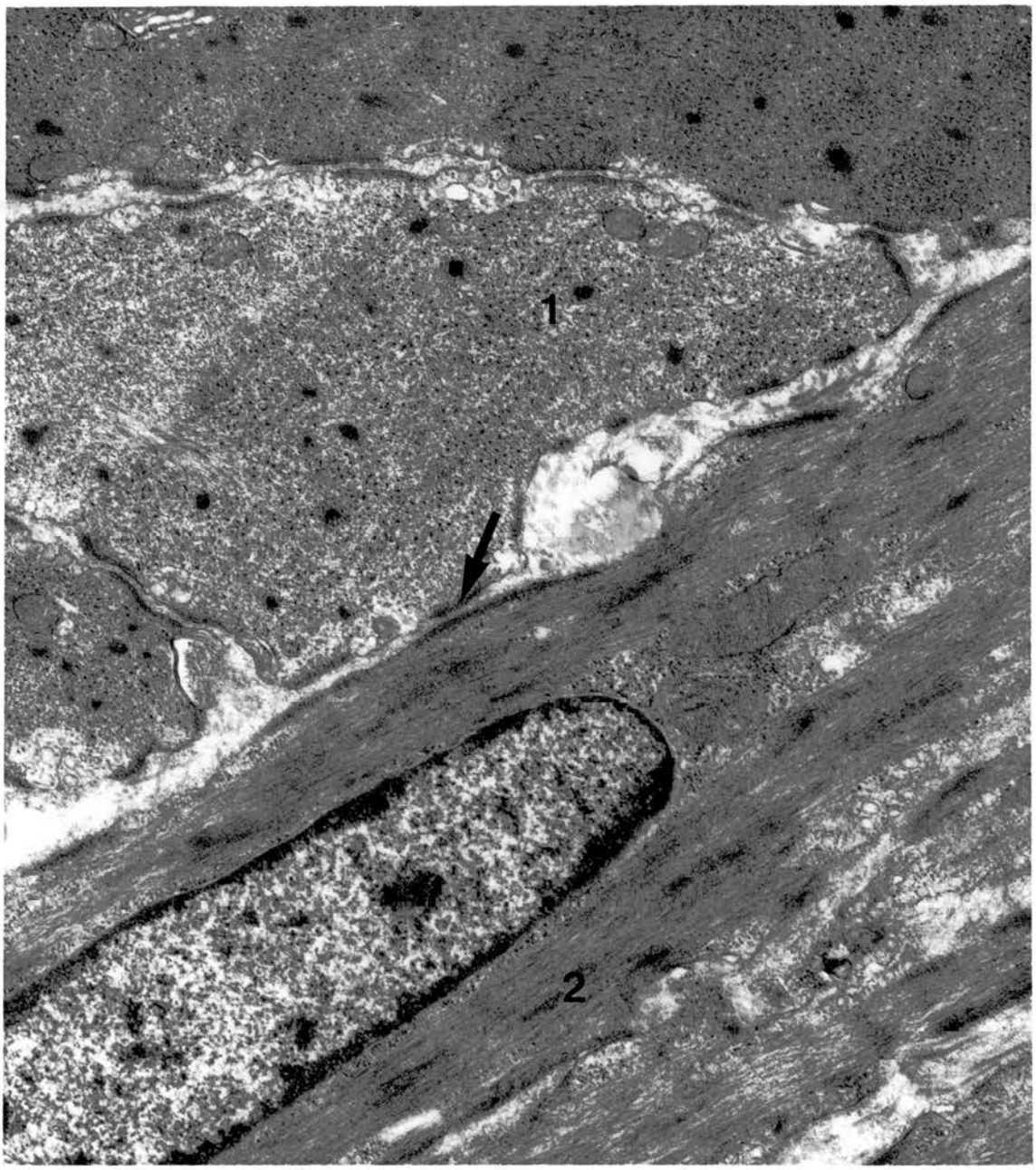
Fig. 17. Transmission electron micrograph of the circular muscle layer of the ileum 5 mm from the ileo-caeco-rectal junction. The muscle cells of the outer circular portion (1) are in some places apposed (arrow) to the cells of the outer longitudinal layer (2). X 25000.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intercellular gap being reduced to 40-50 nm (Fig. 17).

The muscle in the wall of the caecum and rectum 5 mm from the ileo-caeco-rectal junction consisted of three layers including the muscularis mucosae (inner longitudinal muscle layer), the circular muscle layer and the outer longitudinal muscle layer. The inner portion of the circular muscle layer was found to be absent in the caeca and rectum (Fig. 18).

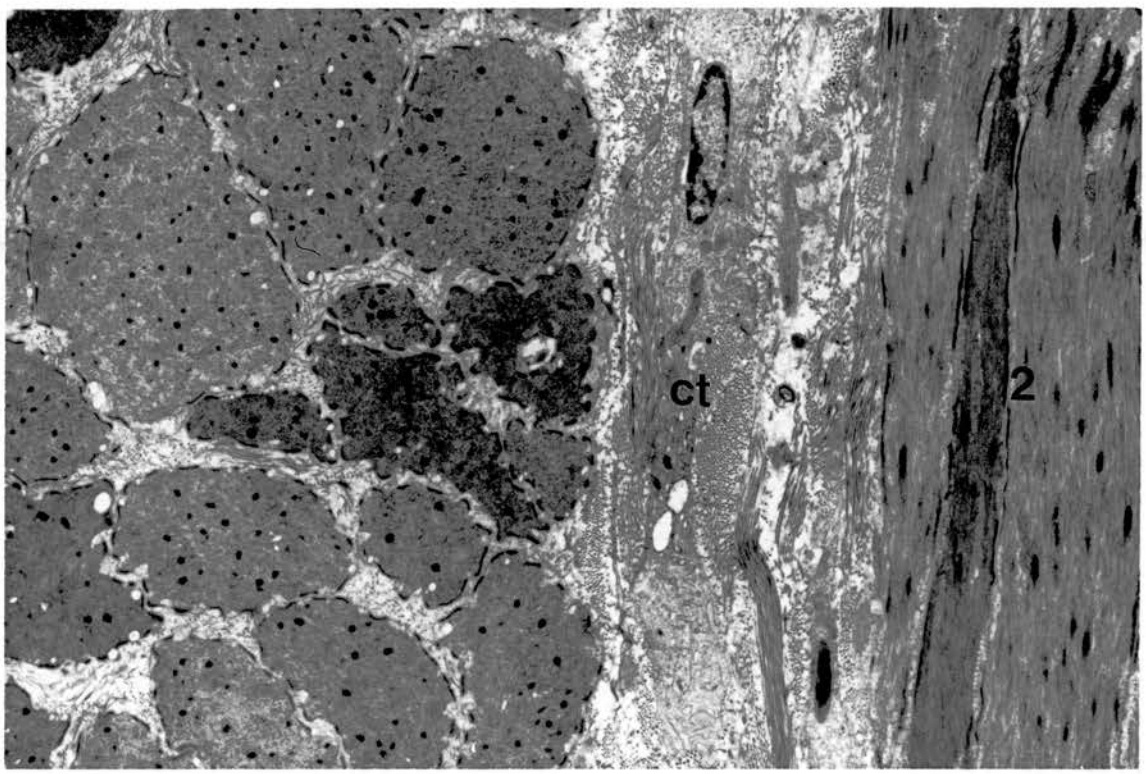
The muscle layer in the ileal papilla was composed only of the muscularis mucosae and the circular layer, the outer longitudinal muscle layer being absent. Furthermore there was no evidence of the cells which were characteristic of the inner portion of the circular layer (Fig. 19). The muscle layer around the caecal orifice was composed of the muscularis mucosae, the circular layer and the longitudinal layer.

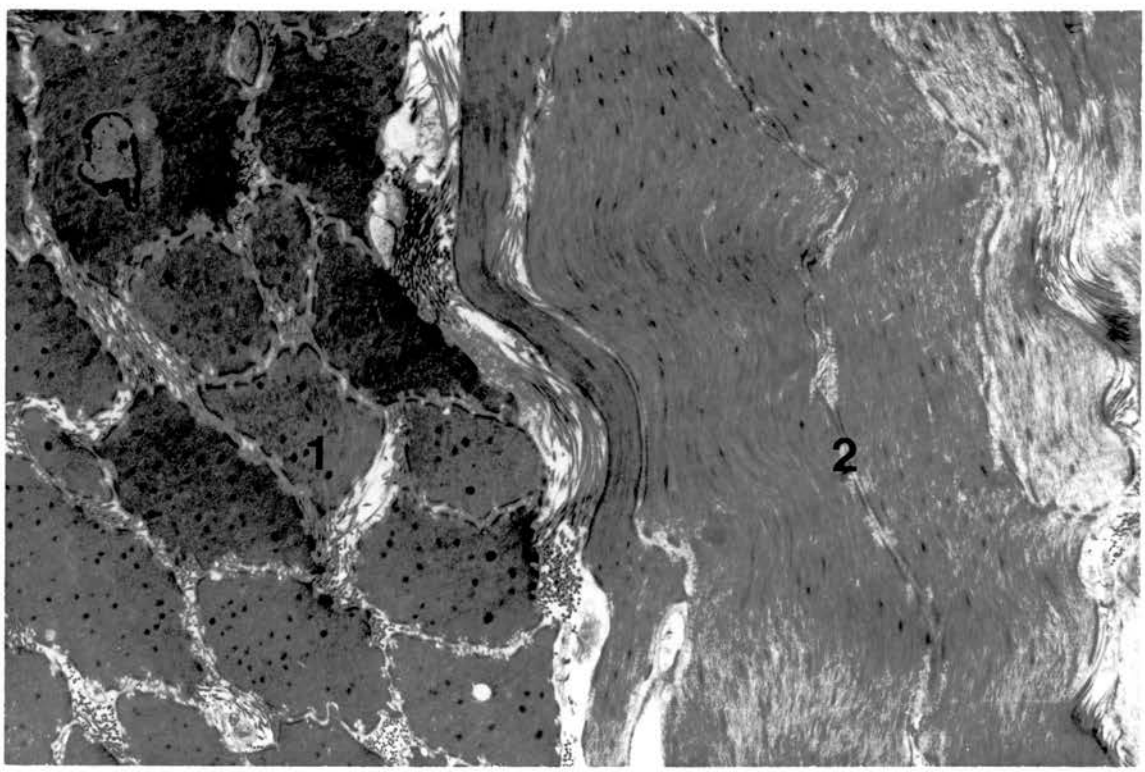
(b) Muscle cells.

The profiles of the muscle cells varied in size due to the fact that their nucleated portions were thicker than their two tapering ends. Generally however, they were oval in cross-section, and somewhat flattened and irregular in outline. The muscle cells of the inner longitudinal layer in all the areas investigated were grouped into small, distinct bundles, the diameter in the nuclear portions being about 3-3.5 μm thick. The muscle cells of the inner portion of the circular layer

Fig. 18. Transmission electron micrograph of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction. The muscularis mucosae (2) is separated from the single circular muscle layer (1) by a thick connective tissue layer (ct). At this level the inner portion of the circular layer is absent. X 7000.

Fig. 19. Transmission electron micrograph of the muscle layer at the base of the ileal papilla which is composed of the muscularis mucosae (2) and the single circular layer (1). The inner portion of the circular layer is absent and the outer longitudinal layer does not extend into the papilla. X 7000.





o

in the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction were small and electron-dense and had a diameter of about 3.5-3.8 μm. They were thin and irregular in outline (Fig. 15). The muscle cells of the outer portion of the circular layer were pale and thick and had a regular outline (Fig. 15). They were not clearly grouped into bundles as they were at the base of the ileal papilla, around the caecal orifice and in the caecum and rectum 5 mm from the ileo-caeco- rectal junction. They had a diameter of 4-4.8 μm. Some cells of the circular layer, particularly in the inner portion of the layer, tended to branch at their tapering ends. The muscle cells of the outer longitudinal layer had a diameter of about 2.8-3.5 μm and were grouped into small bundles.

The nucleus of the muscle cells was usually large and was either round, oval or fusiform in shape with an irregular indented outline. The nucleolus was prominent. Some cells had two nucleoli. The nuclear heterochromatin was usually in the form of a wide band around the periphery of the nucleus, occasional clumps of heterochromatin being observed within the light nucleoplasm (Fig. 20).

The main part of the cell was occupied by myofilaments (Figs. 21, 22) which were orientated parallel to the long axis. Thin, longitudinal myofilaments measuring 4-6 nm in diameter were usually observed. Thick myofilaments measuring 12-15 nm in diameter were easily identified in most preparations by their size and irregular outline. Intermediate myofilaments, 7-11 nm in diameter, were

Fig. 20. Transmission electron micrograph of a cross-section of a muscle cell in the rectum 5 mm from the ileo-caeco-rectal junction. The large nucleus has an irregular outline. A prominent nucleolus (n) is present in the light nucleoplasm. The nuclear heterochromatin is in the form of a band around the periphery of the nucleus. Around the nucleus are scattered free ribosomes (arrows). Dense bodies are indicated by clear arrows. db, dense band. X 30000.

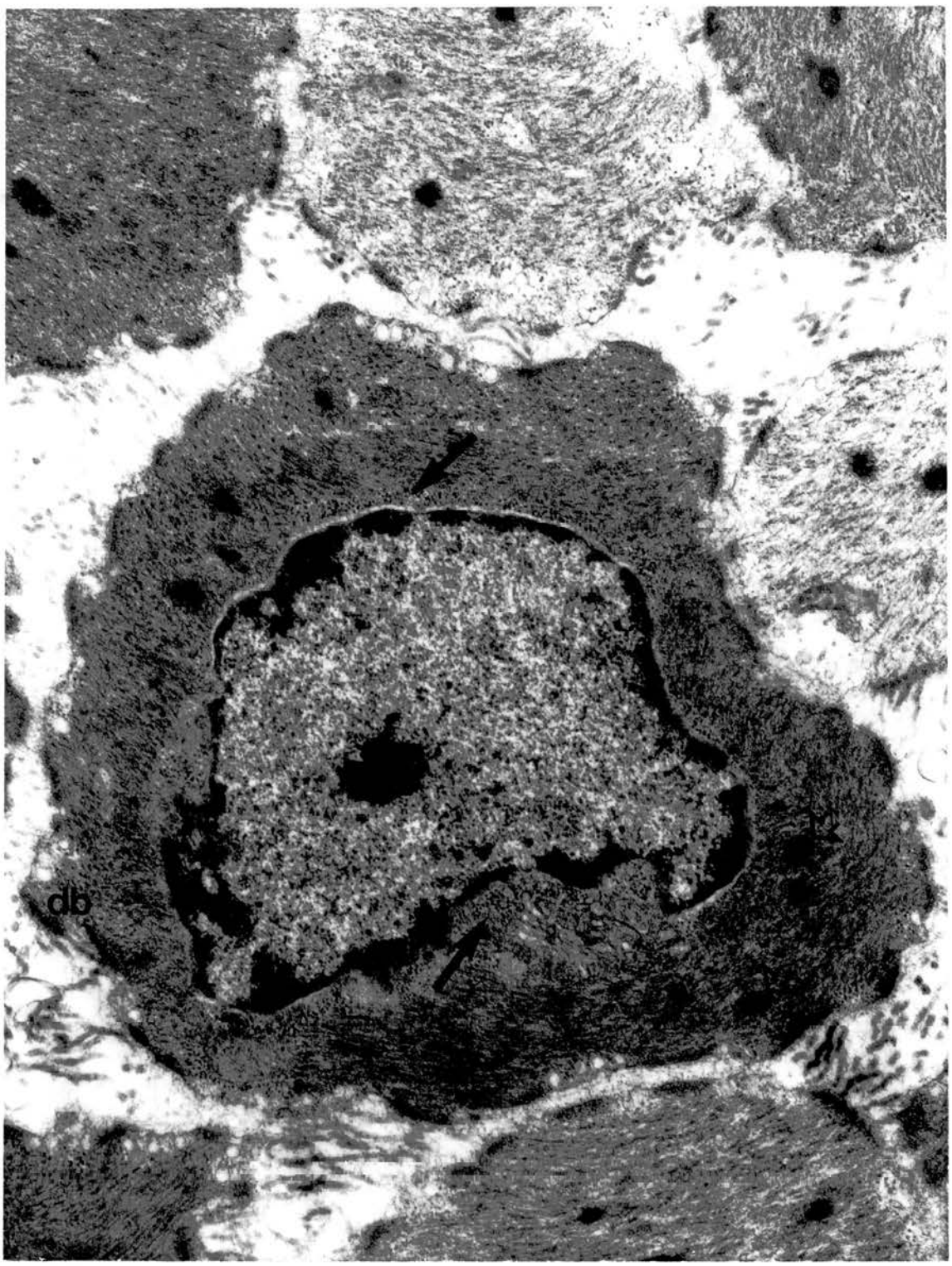
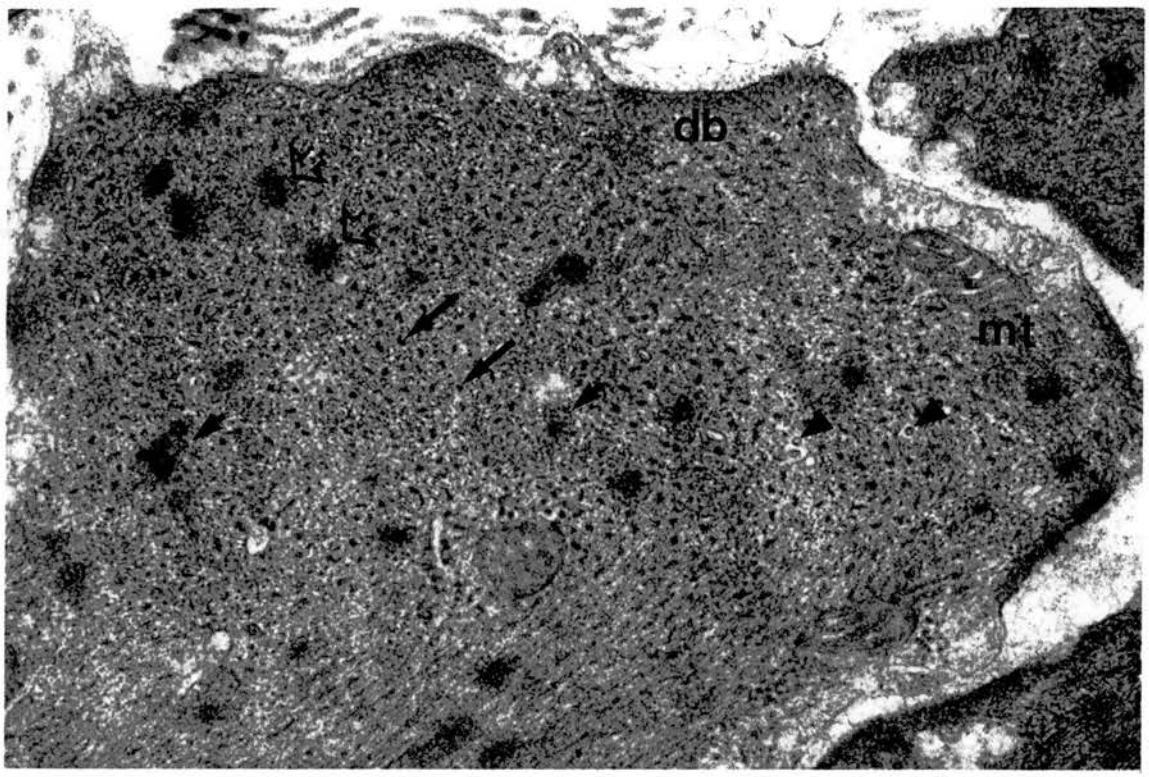

db

Fig. 21. Transmission electron micrograph of a cross-section of a muscle cell at the base of the ileal papilla. The cytoplasm of the cell is occupied by myofilaments. Thick myofilaments (long arrows) are large and have an irregular outline and are scattered throughout the cytoplasm. Thin myofilaments (short arrows) are associated with the dense bodies (clear arrow). Several microtubules (arrowheads) are present between and parallel to the myofilaments. db, dense bands; mt, mitochondria. X 60000.

Fig. 22. Transmission electron micrograph of a longitudinal section of a muscle cell at the caecal orifice showing thick (long arrows), intermediate (short arrows) and thin (arrowheads) myofilaments running parallel to the long axis of the cell. X 83000.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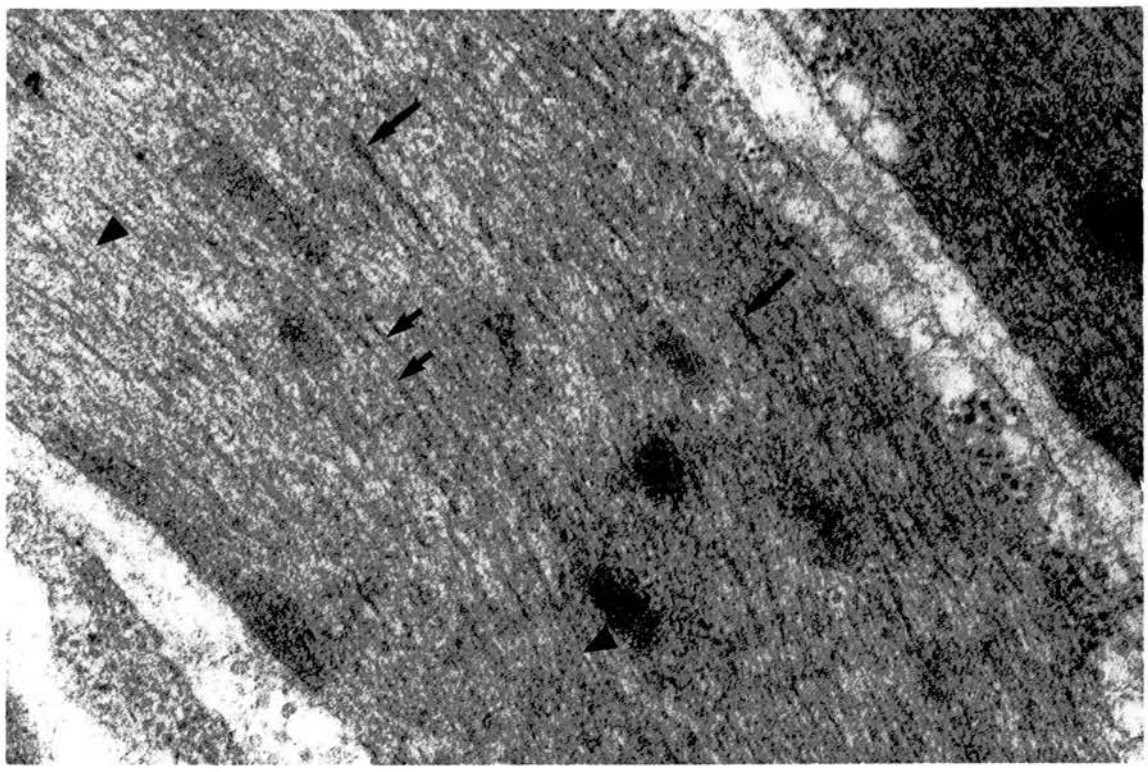


seen in some preparations.

Dense bodies, a characteristic feature of smooth muscle cells, were scattered among the myofilaments. They were numerous, elongated, electron-dense, and nearly circular in cross-section. They were associated with the thin myofilaments (Fig. 23). Dense bands were wide, conspicuous and variable in length. They were attached to a large area of the cell membrane (Fig. 23).

The Golgi complex (Fig. 24) was usually located close to the nucleus and was frequently extensive. It consisted of flattened, occasionally fenestrated cisternae and small electron-lucent vesicles.

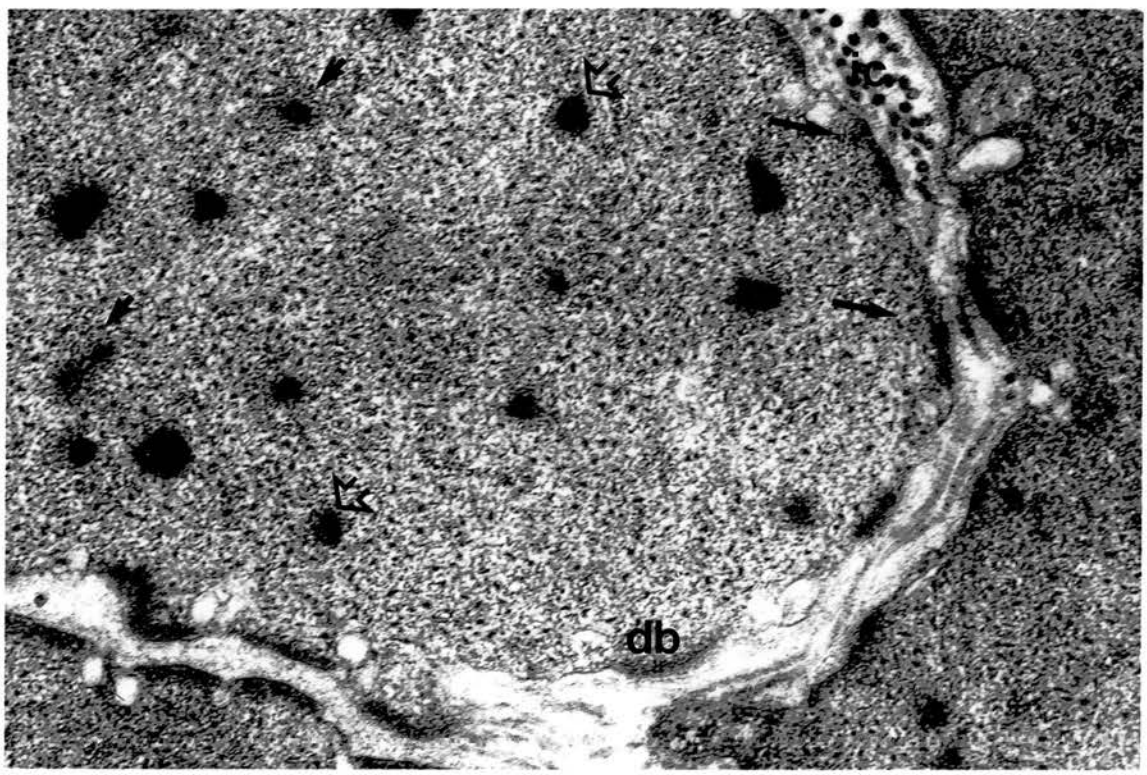
A relatively large number of elongated mitochondria (Fig. 25) with their long axis parallel to the myofilaments were present. They were mainly situated near the nuclear poles or beneath the cell membrane, and occasionally they were distributed singly among the myofilaments. Within the mitochondria the transverse cristae were embedded in a granular matrix. The cristae in some mitochondria were irregularly arranged (Fig. 25).

The plasma membrane of the muscle cells was lined with numerous regular vesicles (caveolae) (Fig. 26), which were oval and arranged in rows parallel to the long axis of the cell. They measured about 60 nm in diameter.

The smooth endoplasmic reticulum (sarcoplasmic reticulum) was commonly observed associated with the caveolae and cell membrane in the form of tubules, cisternae and sacs arranged as a lace-like network spreading among the

Fig. 23. Transmission electron micrograph of a cross-section of a muscle cell in the ileum 5 mm from the ileo-caeco-rectal junction. Dense bodies (clear arrows) are oval and electron dense, and are associated with thin myofilaments (short arrows). Dense bands (db) are wide, variable in length, and are attached to a large area of the cell membrane. They are associated with the thin myofilaments (long arrows). ic, intercellular space. X 44000.

Fig. 24. Transmission electron micrograph of a muscle cell at the base of the ileal papilla. The Golgi complex (g) is extensive and consists of flattened cisternae and small electron-lucent vesicles. mt, mitochondria. X 55000.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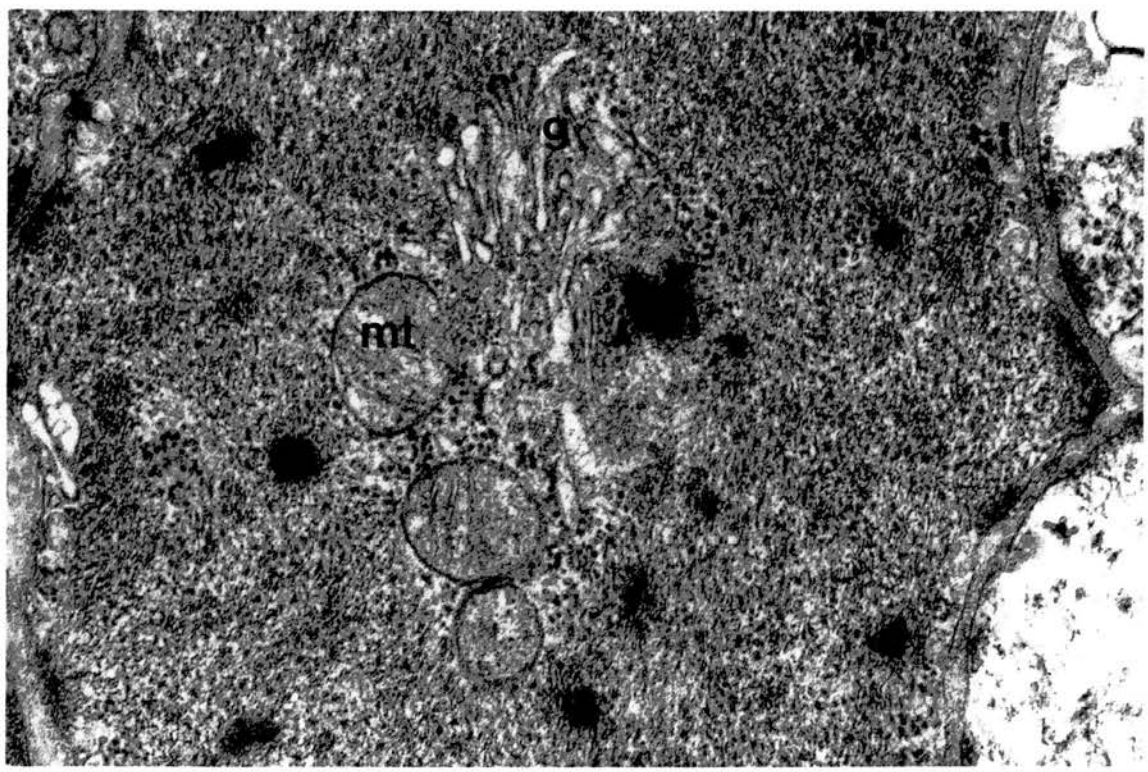
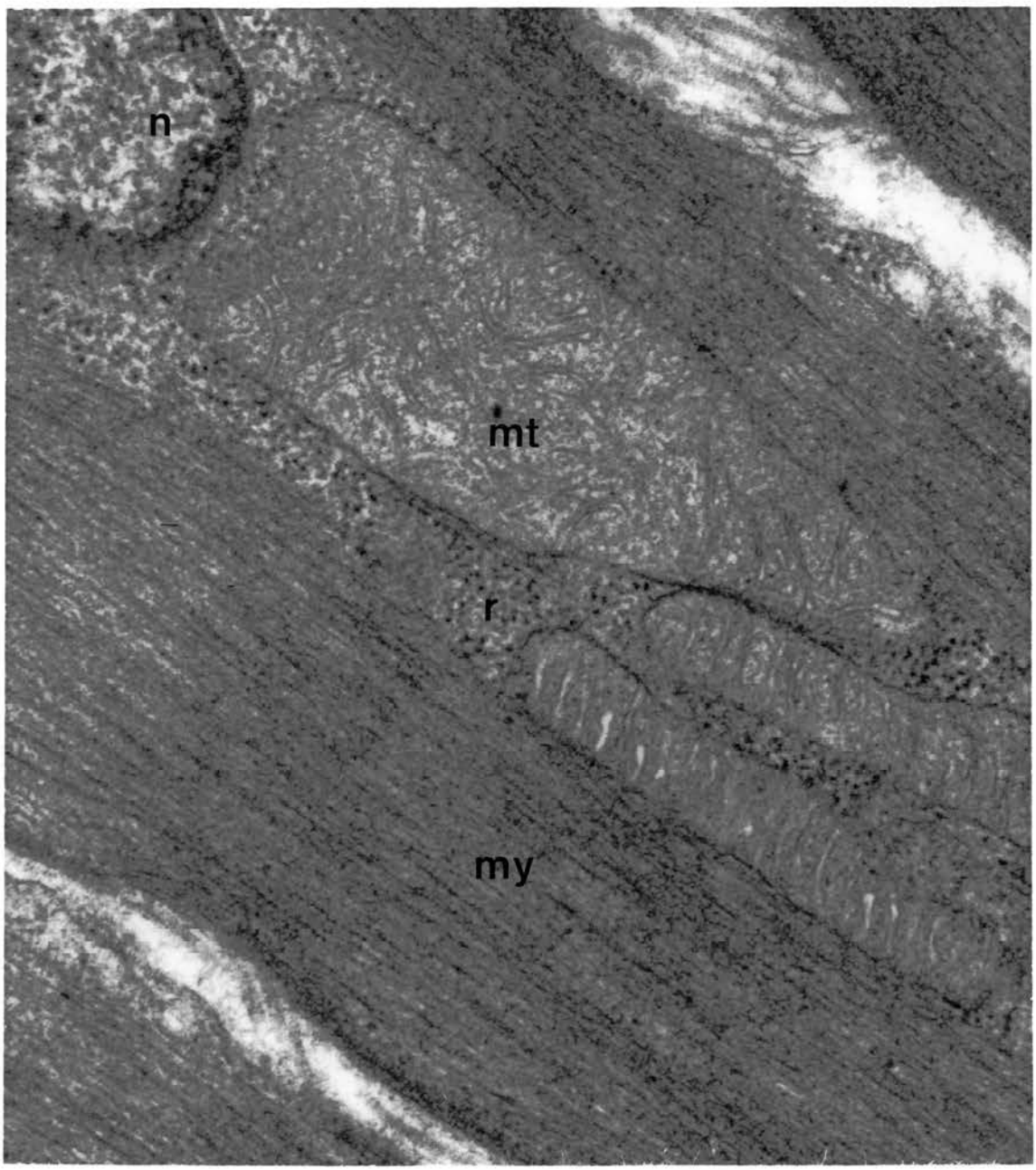

g
mt

Fig. 25. Transmission electron micrograph of a longitudinal section of a muscle cell at the caecal orifice. The mitochondria (mt) have irregular cristae and are situated at the nuclear pole with their long axis parallel to the myofilaments (my). n, nucleus; r, free ribosomes. X 73000.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my

caveolae (Fig. 26) or in the form of long tubules running parallel to the long axis of the cell (Fig. 27). The smooth endoplasmic reticulum did not extend into the centre of the cell.

Amongst the other cytoplasmic contents were ribosomes, which were either free and observed around the nucleus, at the nuclear poles and close to the cell membrane (Figs. 20, 25), or associated with the membranes of the rough endoplasmic reticulum (Fig. 28) in the centre of the cell where it lay close to the mitochondria. Several microtubules measuring 15-20 nm in diameter coursed through the cytoplasm parallel to the myofilaments (Fig. 21). The periphery of each cell was surrounded by a basal lamina about 6-10 nm thick except at the level of the cell junction where it was very thin and ill-defined (Fig. 29). Outside the basal lamina collagen fibres were seen as close as 20-25 nm. Some cell profiles showed a centriole lying close to the nucleus (Fig. 30).

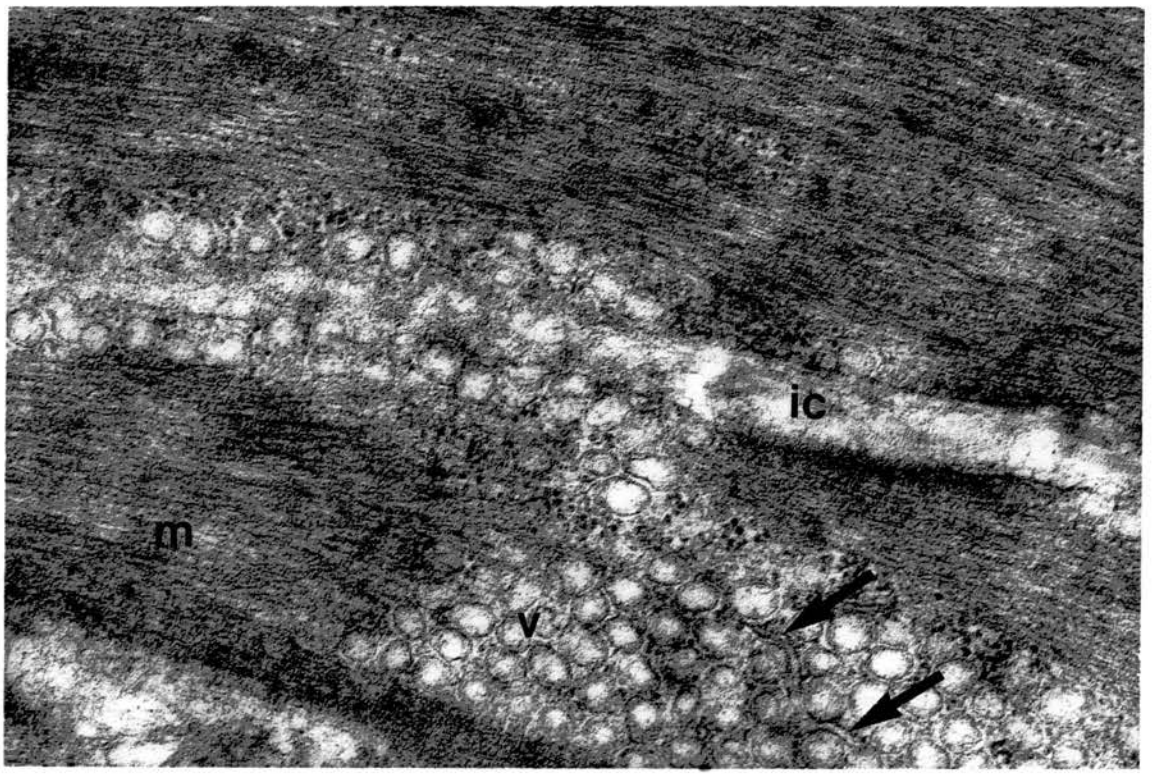
(c) Cell junctions.

The periphery of each muscle cell was completely surrounded by a basal lamina (Fig. 29) and there was no continuity between the cytoplasm of adjacent cells. Two types of intercellular junction between the muscle cells were observed.

In all preparations a small number of nexuses (gap junctions) (Fig. 31) were

Fig. 26. Transmission electron micrograph of muscle cells (m) in the rectum 5 mm from the ileo-caeco-rectal junction. The membrane of the muscle cells is lined with numerous, regular vesicles or " caveolae " (v). The smooth endoplasmic reticulum is distributed among the caveolae forming a lace-like network (arrows). ic, intercellular space. X 58500.

Fig. 27. Transmission electron micrograph of a muscle cell in the caecum 5 mm from the ileo-caeco-rectal junction. The smooth endoplasmic reticulum is in the form of a long tubule (arrows) running among the caveolae (v) parallel to the long axis of the cell. X 67500.


ic
m
v

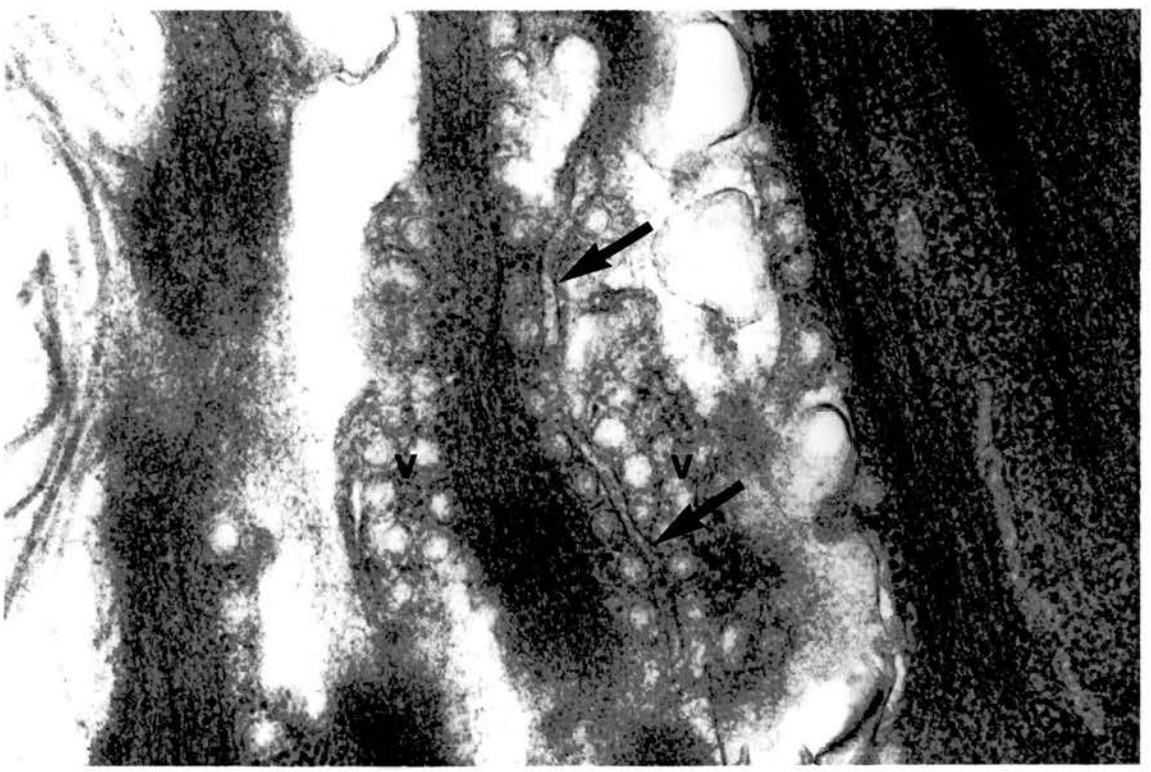
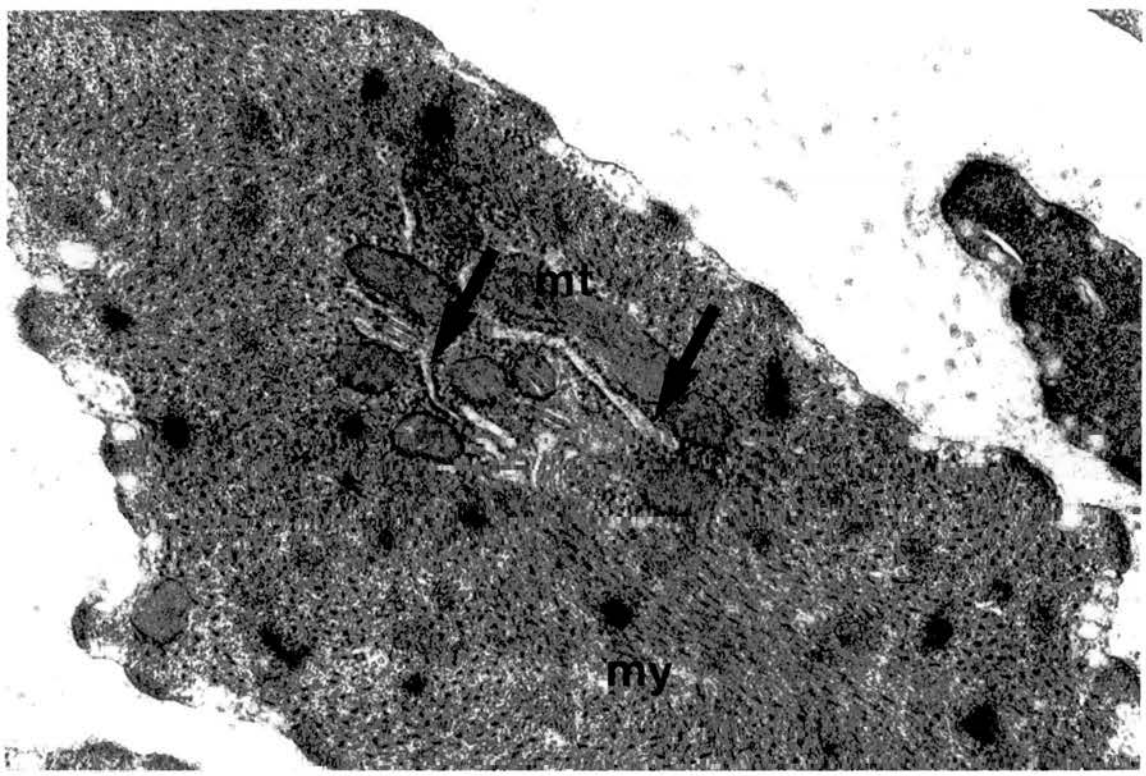

v
v

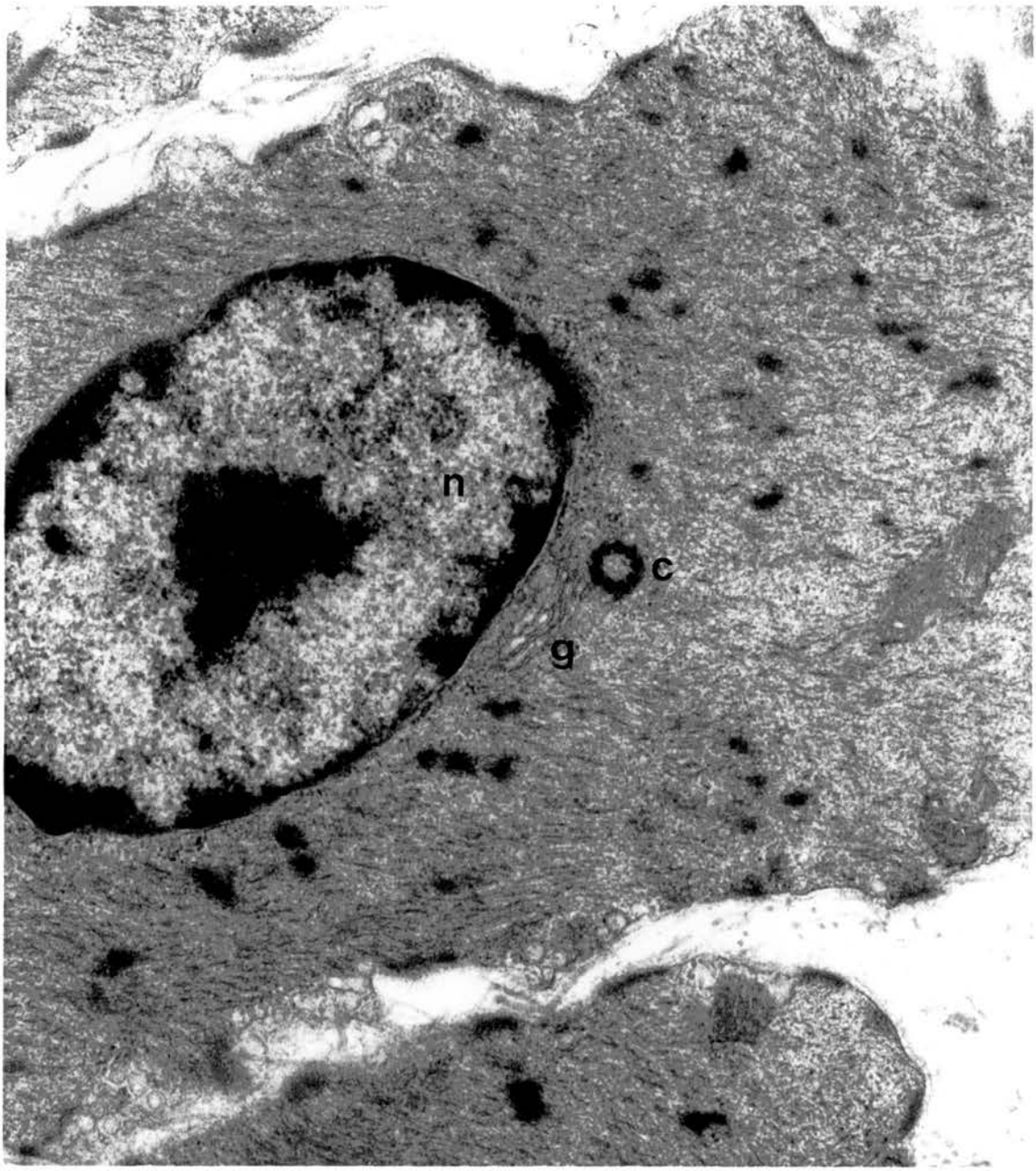
Fig. 28 . Transmission electron micrograph of a muscle cell at the caecal orifice showing the rough endoplasmic reticulum (arrows) situated in the centre of the cell and lying close to the mitochondria (mt). my, myofilaments. X 39500.

Fig. 29. Transmission electron micrograph of muscle cells at the base of the ileal papilla. The muscle cells are surrounded by a basal lamina (large arrows) which becomes ill-defined at the level of the cell junction (small arrow). cf, collagen fibres. X 52500.


mt
my


cf

Fig. 30. Transmission electron micrograph of a muscle cell in the ileum 5 mm from the ileo-caeco-rectal junction showing that the centriole (c) and Golgi complex (g) are situated close to the nucleus (n). The nucleus is surrounded by numerous free ribosomes. X 35500.

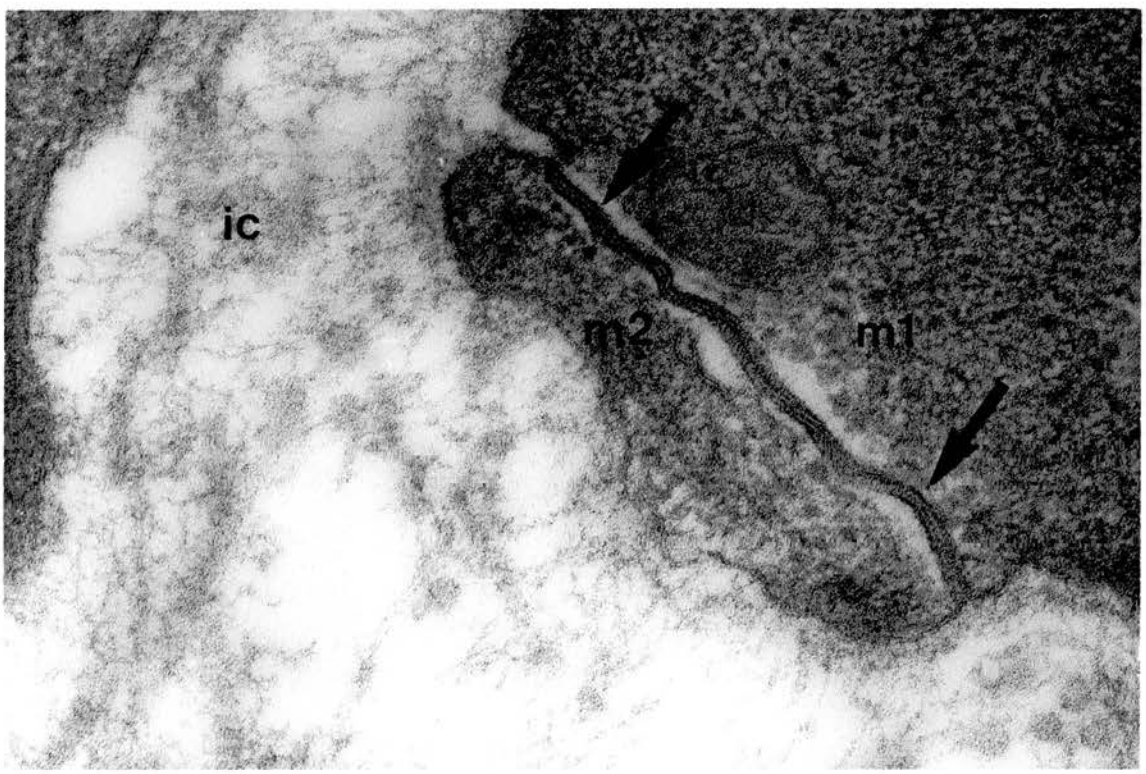

n
c
g

seen between the muscle cells of the circular layer. In the montages of the cross-sections of the circular muscle of the ileum, caecum and rectum 5 mm from the ileo-caeco-rectal junction, about 4-5% of the muscle cells showed nexuses. At the base of the ileal papilla and around the caecal orifices the number of the nexuses was increased to about 8-10%. Nexuses were characterised by the close apposition of the adjacent muscle cells membranes, the intercellular gap being reduced to about 2-3 nm (Fig. 31). Sometimes apparent fusion of the apposing cell membranes was observed. Nexuses between finger-like or dome-shaped cell processes and adjacent cell membranes occurred (Figs. 32, 33). Some muscle cells showed two nexuses with the same cell (Fig. 32). Nexuses were very rarely observed between the muscle cells of the outer longitudinal layer at the base of the ileal papilla and around the caecal orifice and they were not seen between the muscle cells of the inner longitudinal layer in all the areas investigated.

The other type of intercellular junction was the intermediate type (Fig. 34) which appeared to be associated with the dense bands of the cytoplasm of adjacent cells. In most cells the dense bands (Fig. 35) matched each other in size and structure. The intercellular gap at this junction was reduced to about 20-40 nm. Sometimes a long intermediate junction (Fig. 36) about 1-2 μm extended the length of the cell. In this long type of junction a thin layer of electron-dense material in the intercellular gap was observed. Intermediate junctions, about 15-20 nm wide, sometimes were seen between a wide protrusion of a muscle cell (Fig. 37) and an adjacent cell in the circular layer and in both the inner and

Fig. 31. Transmission electron micrograph of the circular muscle at the base of the ileal papilla. The two muscle cells (m1 and m2) form a gap junction (arrows). ic, intercellular space. X 115000.

Fig. 32. Transmission electron micrograph of the circular muscle layer at the caecal orifice. A finger-like muscle cell process (mp) makes a junction with an adjacent muscle cell membrane. Note that one of the muscle cells forms two junctions with adjacent muscle cells (arrows). ic, intercellular space. X 56000.


ic
m2
m1

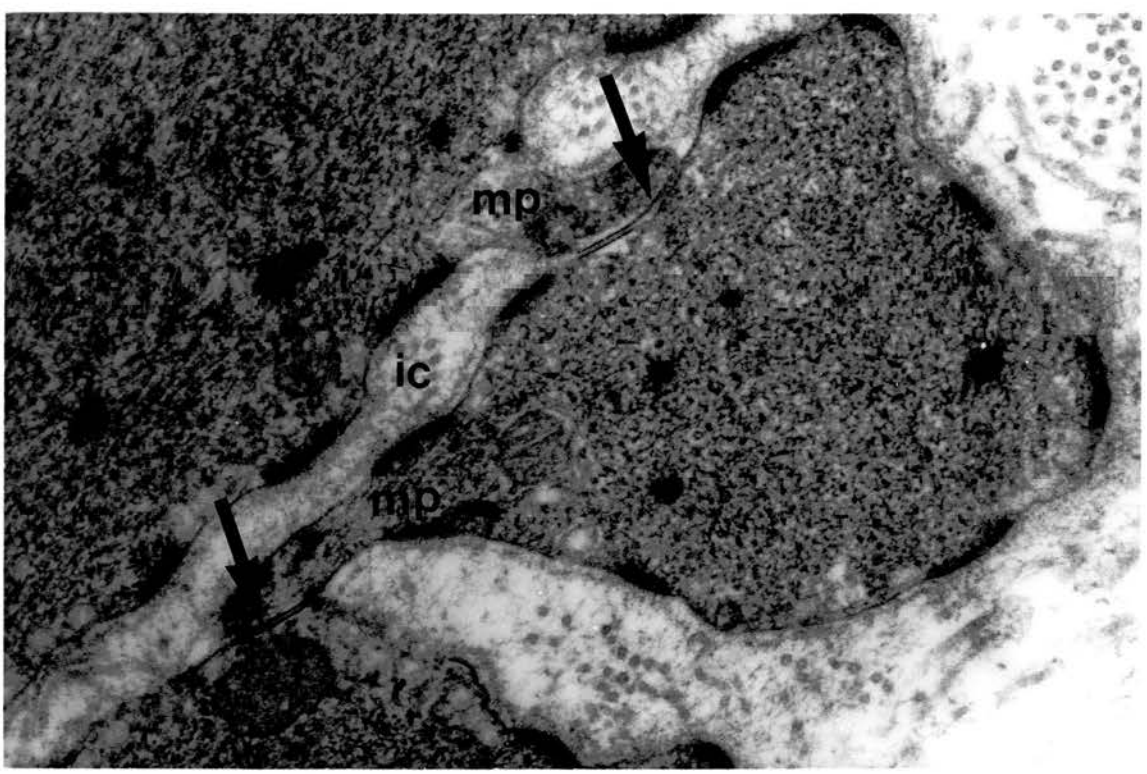

mp
ic
mp

Fig. 33. Transmission electron micrograph of the circular muscle layer at the base of the ileal papilla. A dome-like muscle cell process (mp) forms a gap junction with the membrane of the adjacent muscle cell (arrows). ic, intercellular space; m, muscle cell. X 120000.

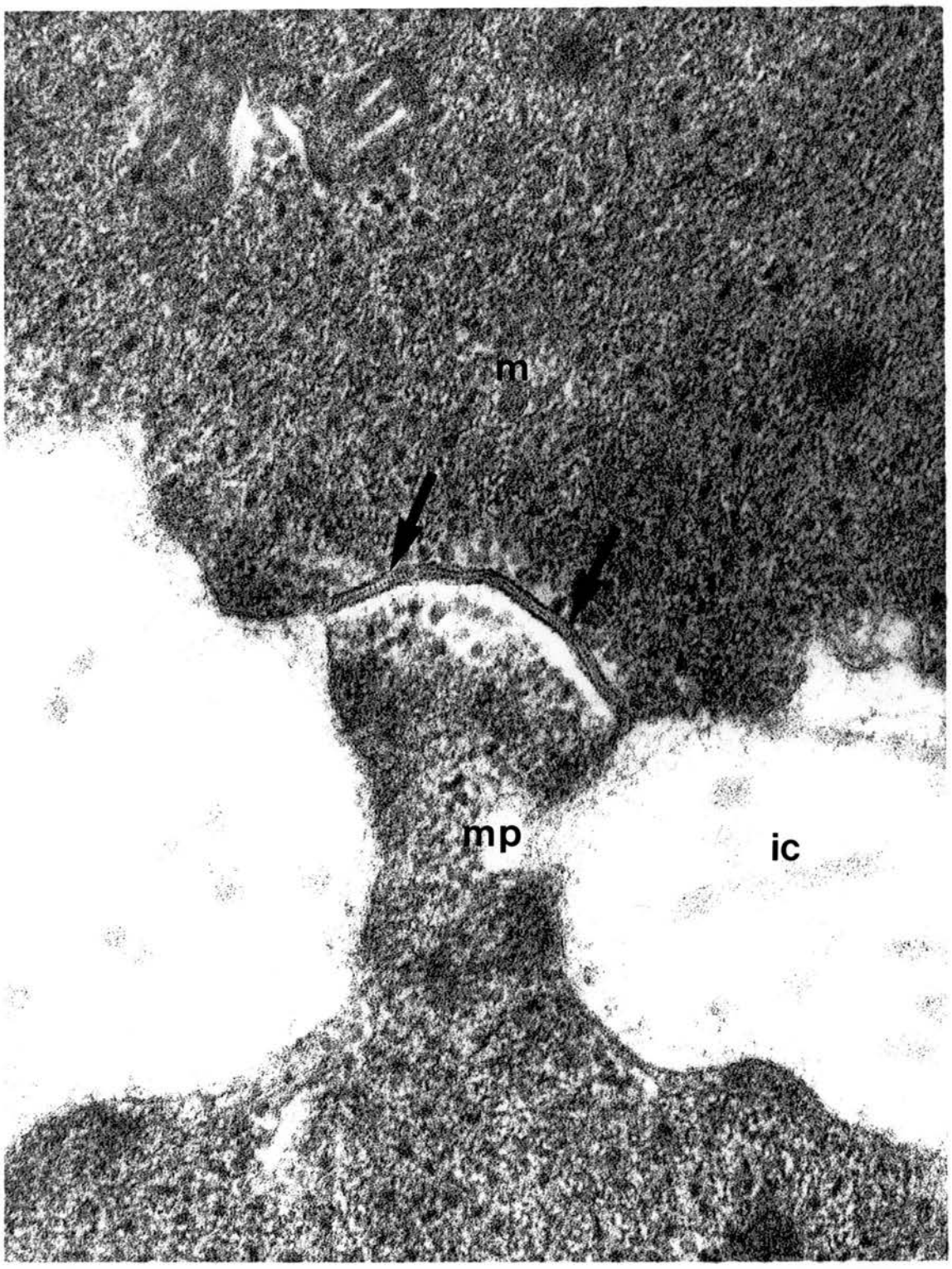
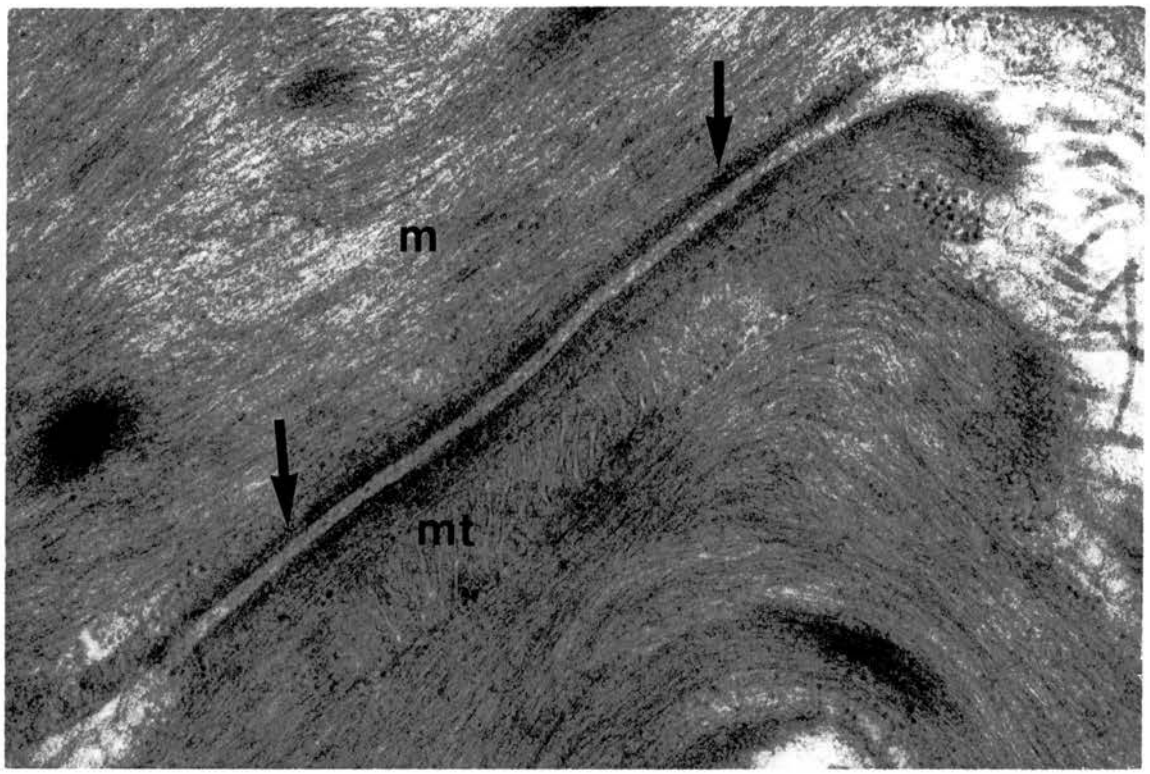

m
mp
ic

Fig. 34. Transmission electron micrograph of the circular muscle layer of the ileum 5 mm from the ileo-caeco-rectal junction showing an intermediate type of intercellular junction (arrows) which appears to be formed between the dense bands of adjacent cells. m, muscle cell; mt, mitochondria. X 50000.

Fig. 35. Transmission electron micrograph of the circular muscle layer of the rectum 5 mm from the ileo-caeco-rectal junction. Many intermediate junctions (arrows) are formed between two of the adjacent muscle cells (m). Medium electron-dense material lies in the intercellular gap. ic, intercellular space. X 51500.


m
mt

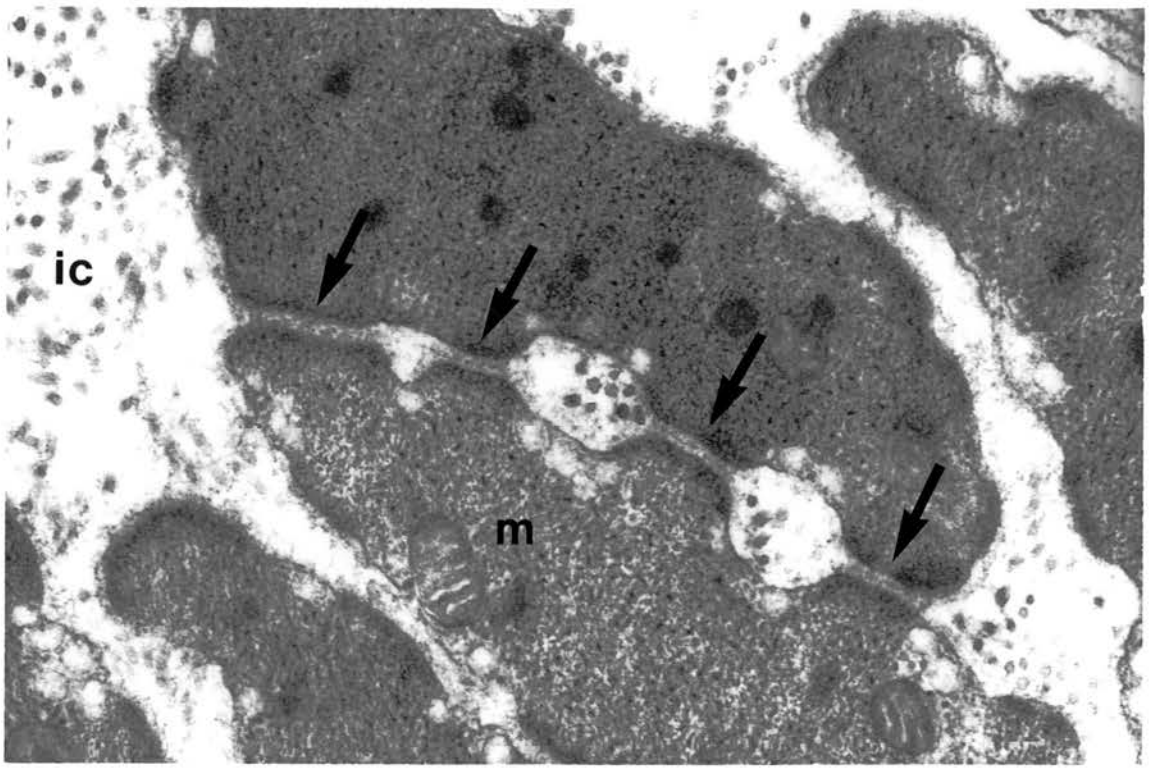
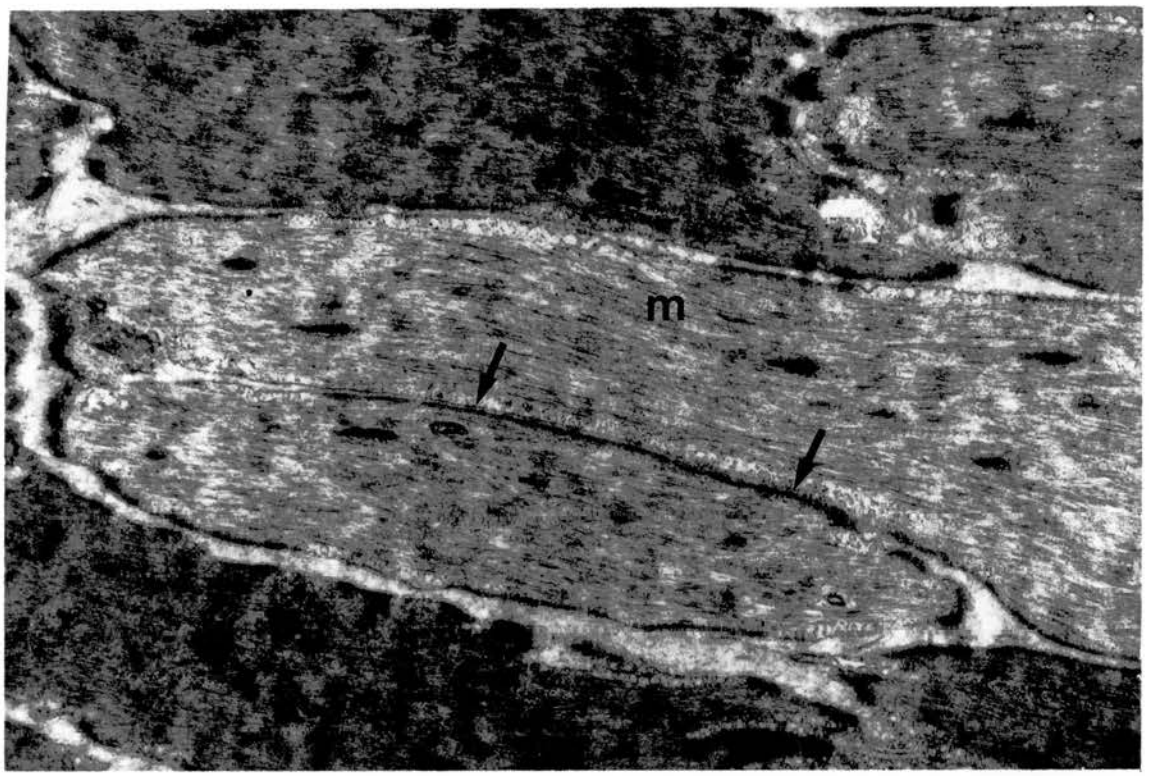
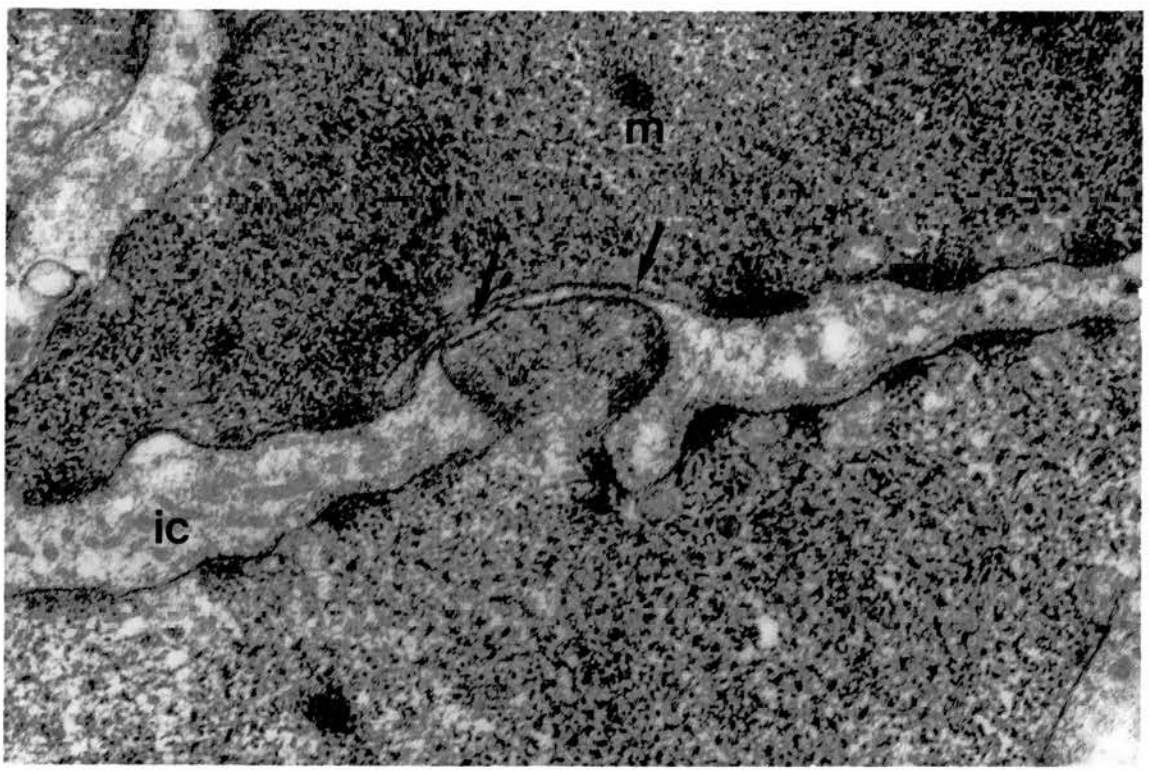

ic
m

Fig. 36. Transmission electron micrograph of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction showing a long intermediate junction (arrows). m, muscle cell. X 16500.

Fig. 37. Transmission electron micrograph of the circular muscle layer at the base of the ileal papilla. A wide protrusion of a muscle cell forms an intermediate junction (arrow) with the body of an adjacent muscle cell. ic, intercellular space; m, muscle cell. X 57500.


m


m
ic

outer longitudinal muscle layers at the base of the ileal papilla and around the caecal orifice. Some cells showed more than one intermediate junction (Fig. 35), the dense bands clearly matching each other and medium electron-dense material being present in the intercellular gap. This type of junction was seen in all muscle layers and in all areas investigated.

(5) Quantitative Observations on the Musculature in the Region of the Ileo-Caeco-Rectal Junction

(a) Measurements of muscle cell length.

The measurement of muscle cell length was based on the formula used by Gabella (1976) and shown below.

$$\text{cell length} = \frac{\text{nucleus average length X 100}}{\text{\% of nucleated cell profiles}}$$

The average nucleus length, the percentage of nucleated muscle cell profiles, and the muscle cell length are shown in Tables 1, 2.

The length of the muscle cell in the muscularis mucosae in all the areas investigated ranged from 243 to 300 μm, the cells at the base of the ileal papilla and around the caecal orifice being longest. The longest muscle cells occurred in the circular layer at the base of the ileal papilla where they were 469 μm in

Table 1

The Length of the muscle cell nuclei (μm) and the percentage of the nucleated muscle cell profiles at the ileo-caeco-rectal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM; longitudinal muscle layer; a, inner circular portion; b, outer circular portion.

Region		Ileum 5mm from junction		Base of ileal papilla		Caecum 5mm from junction		Caecal orifice		Rectum 5mm from junction	
		Nucleus length (µm)	% of nucleated muscle cell profiles	Nucleus length (µm)	% of nucleated muscle cell profiles	Nucleus length (µm)	% of nucleated muscle cell profiles	Nucleus length (µm)	% of nucleated muscle cell profiles	Nucleus length (µm)	% of nucleated muscle cell profiles
	1	21.45	6.82	15.00	6.17	16.25	5.56	14.62	8.34	18.12	6.92
MM	2	14.61	4.19	20.50	6.48	13.50	7.22	20.42	6.19	14.13	6.41
	3	13.20	6.59	17.25	5.05	19.17	7.66	17.23	5.99	16.10	5.58
	1a	16.19	9.37	21.10	5.97	19.11	7.00	18.25	5.70	22.97	5.75
	1b	18.13	5.43								
CM	2a	23.07	8.77	25.60	4.20	15.76	4.69	24.81	6.10	14.59	6.68
	2b	24.35	7.01								
	3a	18.29	7.06	16.94	3.80	23.06	6.20	16.86	2.66	19.76	8.42
	3b	19.90	4.84								
	1	22.70	8.63	18.66	7.19	16.50	8.26	17.59	9.14	16.03	9.51
LM	2	17.15	8.70	16.68	8.32	20.73	7.99	19.61	5.76	21.30	8.00
	3	18.20	6.91	21.74	7.00	17.50	7.06	22.00	8.35	14.15	7.81

Table 2

The length of the muscle cells (μm) at the ileo-caeco-rectal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer; a, inner circular portion; b, outer circular portion.

Region		Ileum 5mm from junction	Base of ileal papilla	Caecum 5mm from junction	Caecal orifice	Rectum 5mm from junction
	1	314.51	243.11	292.26	175.29	261.84
MM	2	348.68	316.35	186.98	329.88	220.43
	3	200.30	341.58	250.26	287.64	275.21
Mean		287.83	300.34	243.16	264.27	252.49
S.D.		± 77.70	± 51.14	± 52.99	± 79.90	± 28.56
	1a	172.78	353.43	273.00	320.17	399.47
	1b	333.88				
CM	2a	263.05	609.52	336.03	406.72	218.41
	2b	347.36				
	3a	259.06	445.78	371.93	633.83	235.03
	3b	411.15				
Mean	a	231.63	469.57	326.98	453.57	264.30
S.D.		± 51.00	±129.69	± 50.08	±161.99	± 65.62
Mean	b	364.13				
S.D.		± 41.27				
	1	263.03	259.52	199.75	192.45	168.55
LM	2	197.12	200.48	259.44	340.45	266.25
	3	263.38	310.57	247.87	263.47	181.17
Mean		241.17	256.85	235.68	265.45	205.32
S.D.		± 38.15	± 55.09	±31.65	±74.02	± 53.14

length, whilst around the caecal orifice they were 453 μm. The shortest cells in the circular layer were about 230 μm in length and occurred in the inner portion of the layer in the ileum 5 mm from the ileo-caeco-rectal junction. The muscle cells of the longitudinal layer had the shortest length and ranged from 205 to 265 μm.

In all three muscle layers there were apparent differences in the length of the muscle cells between those at the base of the ileal papilla and those in the ileum and rectum 5 mm from the ileo-caeco-rectal junction. Apparent differences were also found between the length of the muscle cells around the caecal orifice and those in the caecum and rectum 5 mm from the junction. However, the number of the specimens was too small to establish statistically significant differences (Table 3).

(b) Measurements of muscle cell volume.

The measurement of the volume of the muscle cells was based on the formula used by Gabella (1976) and shown below.

$$\text{cell volume} = \frac{\text{sum of all profile surfaces X nucleus length}}{\text{number of nucleated profiles}}$$

The sum of the muscle cell profile surfaces, the nucleus length, the number of nucleated muscle cell profiles and the volume of the muscle cells are shown

Table 3

The mean of the muscle cell length (μm) at the ileo-caeco-rectal junction of three birds compared with the ileum, caecum and rectum. MM, muscularis mucosa; CM, circular muscle; LM, longitudinal muscle.

Region	MM	Statistical significance (t-test)	CM	Statistical significance (t-test)	LM	Statistical significance (t-test)
Ileum 5mm from junction	287.83	t=0.23 not significant	364.13	t=1.34 not significant	241.17	t=0.40 not significant
Base of ileal papilla	300.34	t=1.14 not significant	469.57	t=2.45 not significant	256.85	t=1.17 not significant
Rectum 5mm from junction	252.49	t=0.24 not significant	264.30	t=1.88 not significant	205.32	t=1.14 not significant
Caecal orifice	243.16	t=0.38 not significant	453.57	t=1.29 not significant	265.45	t=0.64 not significant
Caecum 5mm from junction	243.16		326.98		235.68	

in Tables 1, 4, 5.

In the five areas investigated the volume of the muscle cells of the muscularis mucosae ranged from 1133 to 1762 μm3 whilst those of the circular layer ranged from 2045 to 3027 μm^3. The smallest volume in the circular layer, 1284 μm^3, occurred in the inner portion in the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction. The muscle cells of the longitudinal layer had the smallest volume (1020-1255 μm^3).

At the base of the ileal papilla and around the caecal orifices the muscle cells had the largest volume in all the areas investigated. In the three layers there was an apparent difference in the volume of the muscle cells between those at the base of the ileal papilla and those in the ileum and rectum 5 mm from the ileo-caeco-rectal junction. A similar difference was found between the volume of the muscle cells around the caecal orifice and in the caecum and rectum 5 mm from the junction. However, the number of specimens was too small to establish statistically significant differences (Table 6).

(6) Ultrastructure of the Nerve Bundles in the Region of the Ileo-Caeco-Rectal Junction.

(a) Nerve bundles.

Nerve bundles and vesiculated axon profiles were observed in all muscle

Table 4

The total surface area (μm^2) and the total number of nucleated muscle cell profiles at the ileo-caeco-rectal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer; a, inner circular portion; b, outer circular portion.

Region		Ileum 5mm from junction		Base of ileal papilla		Caecum 5mm junction		Caecal orifice		Rectum 5mm from junction	
		Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles
	1	1874.23	25	2857.68	25	4307.14	44	5936.39	52	4108.01	61
MM	2	1849.92	18	2368.85	33	3919.27	39	3640.95	53	3176.00	44
	3	1346.43	14	4749.70	39	3332.61	39	4640.60	41	2807.06	39
	1a	2050.11	28	3330.96	28	2418.63	21	1867.96	13	3420.67	36
	1b	2979.20	25								
CM	2a	1099.39	19	3293.09	25	1951.95	15	2191.90	18	2653.18	20
	2b	2683.34	26								
	3a	1605.31	22	2645.16	14	1931.22	18	2812.07	19	2753.09	27
	3b	2341.47	17								
	1	2125.70	44	2322.05	38	2997.43	52	3395.10	54	3482.61	51
LM	2	2927.08	50	2196.33	33	1992.60	37	4002.15	61	2875.18	62
	3	3913.40	58	2261.83	33	2443.21	43	3123.27	50	3007.07	42

Table 5

Volume of the muscle cells (μm^3) at the ileo-caeco-rectal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer; a, inner circular portion; b, outer circular portion.

Region		Ileum 5mm from junction	Base of ileal papilla	Caecum 5mm from junction	Caecal orifice	Rectum 5mm from junction
	1	1608.08	1714.60	1590.70	1669.03	1220.28
MM	2	1501.52	1471.55	1356.67	1402.79	1019.92
	3	1269.49	2100.82	1638.10	1950.18	1158.81
Mean		1459.70	1762.32	1528.49	1674.00	1133.00
S.D.		±173.12	±317.33	±150.67	±273.72	±102.64
	1a	1185.40				
	1b	2160.51	2510.12	2200.95	2622.32	2182.57
CM	2a	1334.89				
	2b	2513.05	3372.13	2050.85	3021.17	1935.49
	3a	1334.59				
	3b	2740.90	3200.64	2474.11	2495.34	2017.91
Mean	a	1284.96				
S.D.		± 86.22				
Mean	b	2471.49	3027.63	2241.97	2712.94	2045.32
S.D.		±292.41	±456.30	±214.59	±274.37	±125.80
	1	1096.67	1140.24	951.11	1105.92	1094.63
LM	2	1003.98	1110.15	1116.40	1286.59	987.76
	3	1228.00	1490.07	994.32	1374.24	1038.15
Mean		1109.55	1246.82	1020.61	1255.58	1040.18
S.D.		±112.56	±211.19	±85.72	±136.81	±53.46

Table 6

The mean of the muscle cell volume (μm^3) at the ileo-caeco-rectal junction in three birds compared with the ileum, caecum and rectum. MM, muscularis mucosae; CM, circular muscle; LM, longitudinal muscle.

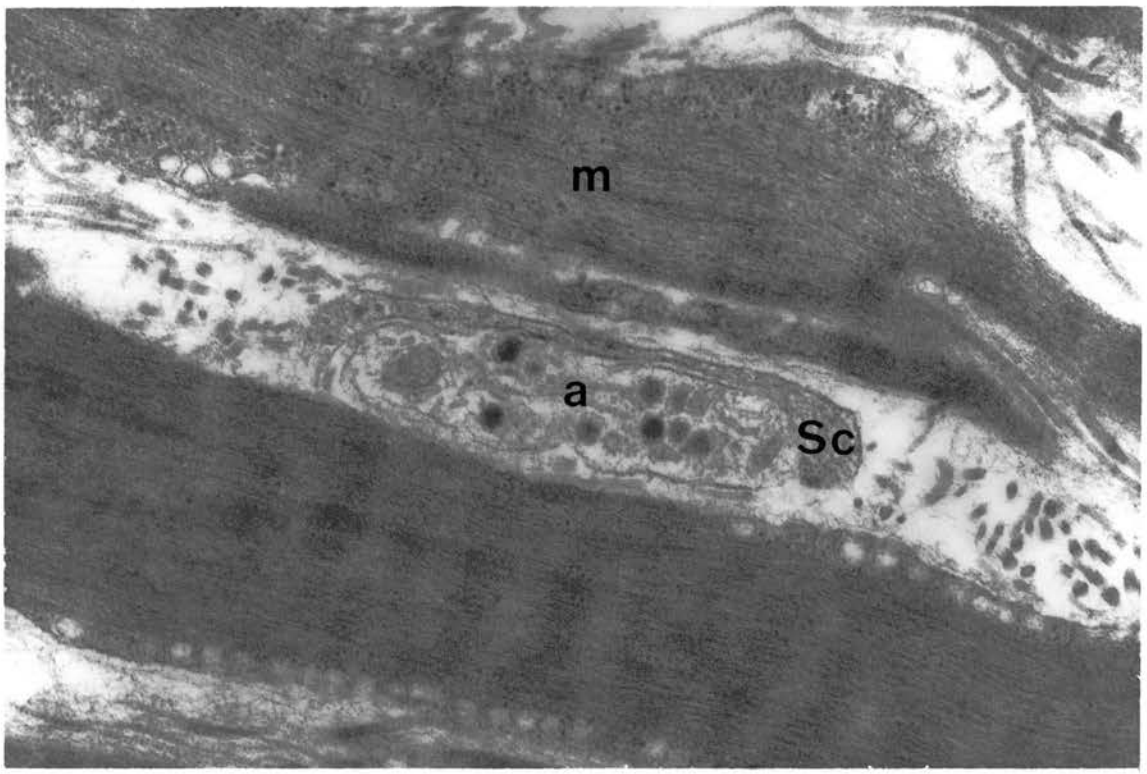
Region	MM	Statistical significance (t-test)	CM	Statistical significance (t-test)	LM	Statistical significance (t-test)
Ileum 5mm from junction	1459.70	t=1.45 not significant	2471.49	t=1.78 not significant	1109.55	t=0.99 not significant
Base of ileal papilla	1762.32	t=3.27 not significant	3027.63	t=3.59 (p<0.05)	1246.82	t=1.64 not significant
Rectum 5mm from junction	1133.00	t=3.21 not significant	2045.32	t=3.83 (p<0.02)	1040.18	t=2.54 not significant
Caecal orifice	1674.00	t=0.80 not significant	2712.94	t=2.34 not significant	1255.58	t=2.52 not significant
Caecum 5mm from junction	1528.49		2241.97		1020.61	

layers. They were rare in both the inner (muscularis mucosae) and outer longitudinal layers (Figs. 38, 39) and were sometimes totally absent in the outer longitudinal layer. In the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction, the innervation consisted of a very few small nerve bundles lying between the muscle cells of the inner portion of the circular layer (Fig. 40), and numerous medium to large-sized nerve bundles in the connective tissue space between the inner and outer portions of the circular layer (Fig. 40). However, the majority of nerve bundles were found between the muscle cells of the outer portion. In the caecum and rectum 5 mm from the ileo-caeco-rectal junction and at the base of the ileal papilla and around the caecal orifice (where there is only a single circular muscle layer) nerve bundles of different sizes were distributed throughout the thickness of the circular layer (Fig. 41). In all muscle layers nerve bundles ran approximately parallel to the long axes of the muscle cells.

All axons were unmyelinated and completely or partly surrounded by Schwann cell cytoplasm (Fig. 42). However, small groups of axons ran for a short distance in close contact to each other without any intervening Schwann cell cytoplasm. The nerve bundles in the circular muscle layer in all the montages of the five areas investigated consisted of 1-100 axons (Figs. 43, 44), the majority of the nerve bundles containing 2-45 axons. The largest nerve bundles had 80-100 axons and were only observed in the circular muscle layer at the base of the ileal papilla and around the caecal orifices.

Fig. 38. Transmission electron micrograph of an axon profile (a) containing granular vesicles between the inner longitudinal muscle cells of the ileum 5 mm from the ileo-caeco-rectal junction. m, muscle cell; Sc, Schwann cell process. X 40000.

Fig. 39. Transmission electron micrograph of an axon profile (a) containing different sizes of granular vesicles between the outer longitudinal muscle cells at the base of the ileal papilla. m, muscle cell; Sc, Schwann cell process. X 40000.


m
a
Sc

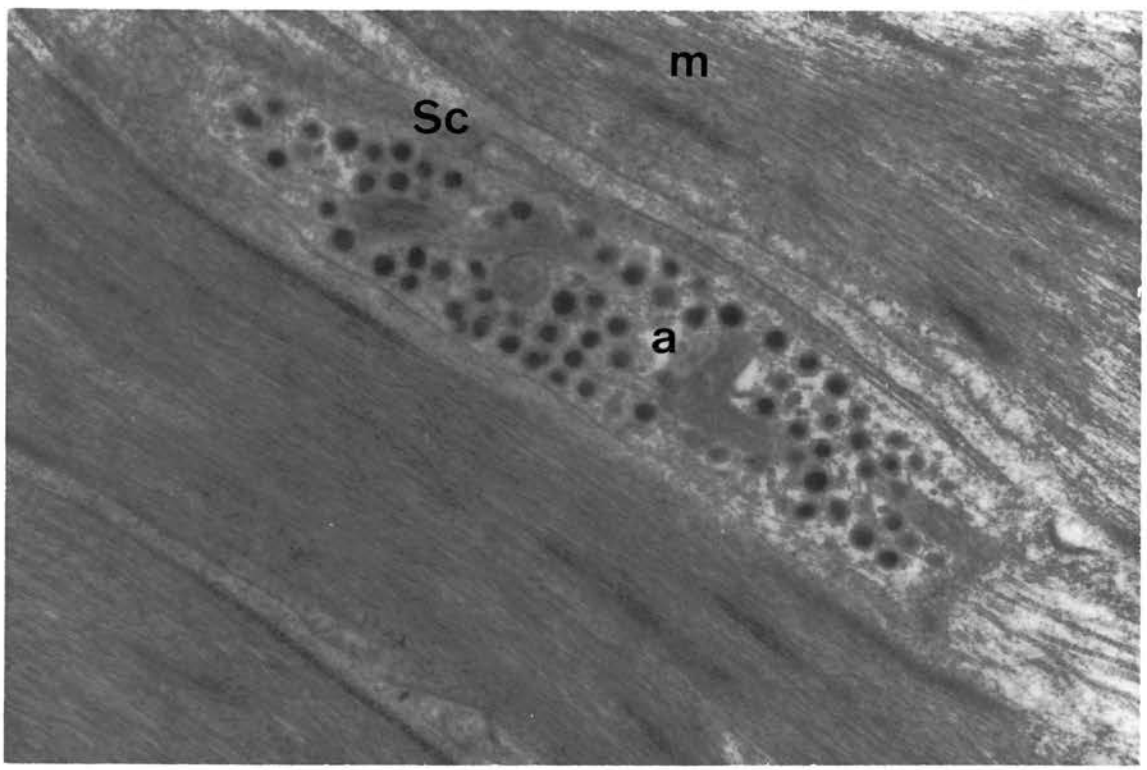

m
Sc
a

Fig. 40. Transmission electron micrograph of a transverse section through the circular muscle layer in the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction. A small nerve bundle (arrow) lies between the muscle cells in the inner portion of the layer (2). A large nerve bundle (NB) is situated in the connective tissue between the inner and outer (1) portions of the circular layer. Note that there are many interstitial cells (IC) associated with this nerve bundle. 3, inner longitudinal muscle layer. X 7500.

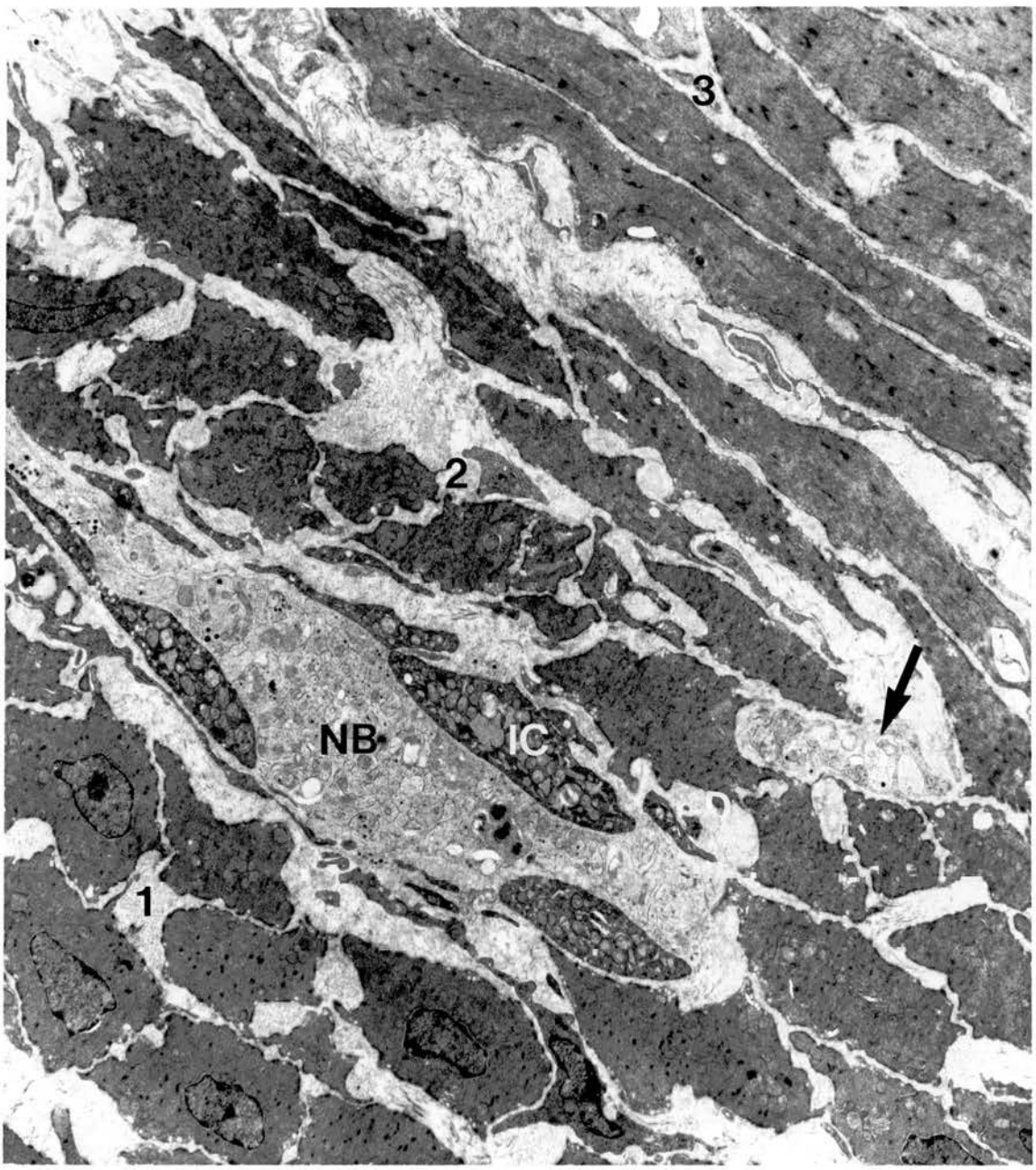

3
2
NB
IC
1

Fig. 41. Transmission electron micrograph of a transverse section through the circular muscle layer around the caecal orifice. Nerve bundles (arrows) of different sizes are distributed throughout the layer. X 6500.

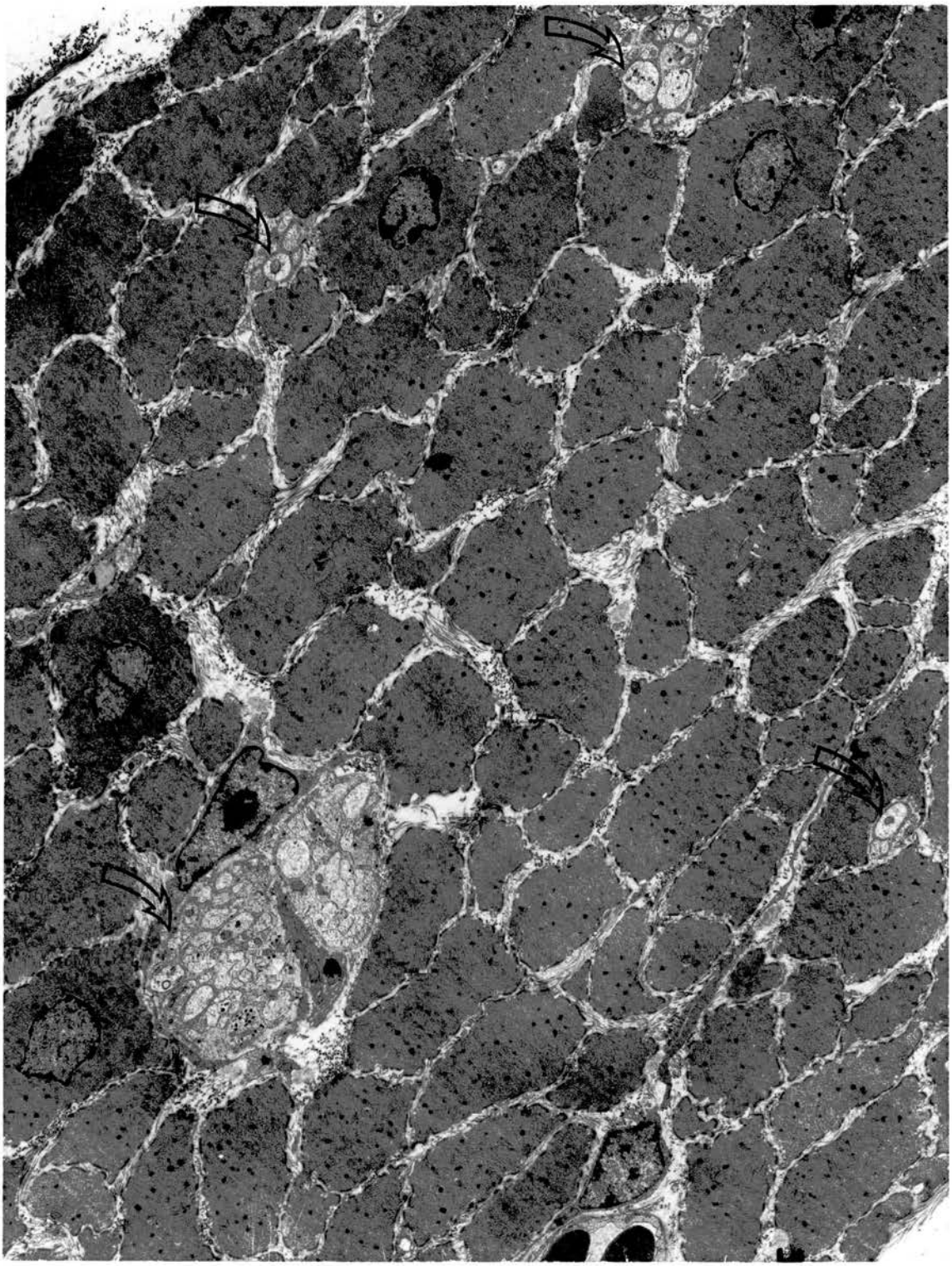


Fig. 42. Transmission electron micrograph of a bundle of axon profiles between the circular muscle cells of the rectum 5 mm from the ileo-caeco-rectal junction. All the axon profiles are completely or partially surrounded by Schwann cell processes (Sc). Axon (a1) contains large granular vesicles while axon (a2) contains agranular vesicles. m, muscle cell. X 80000.

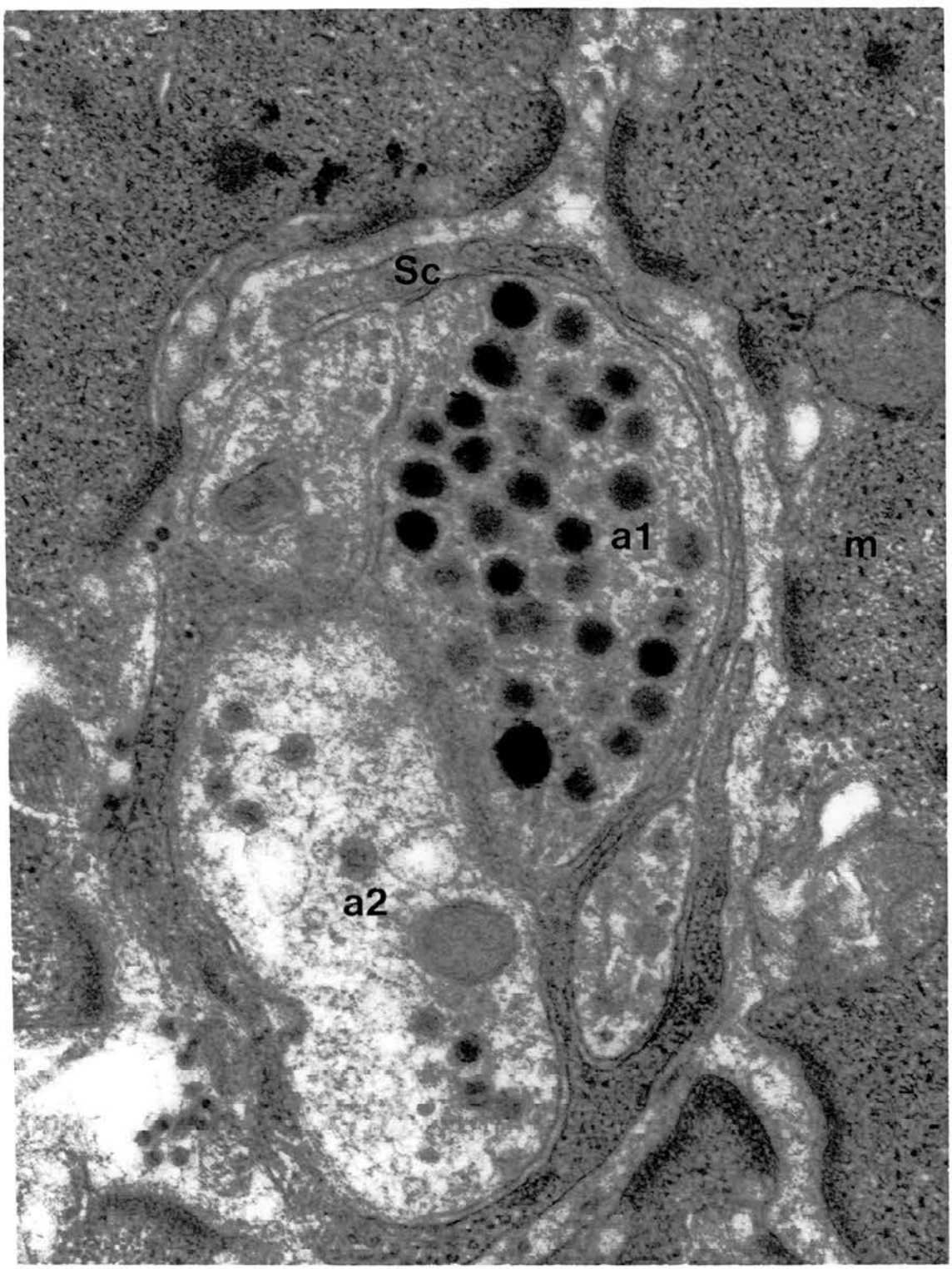
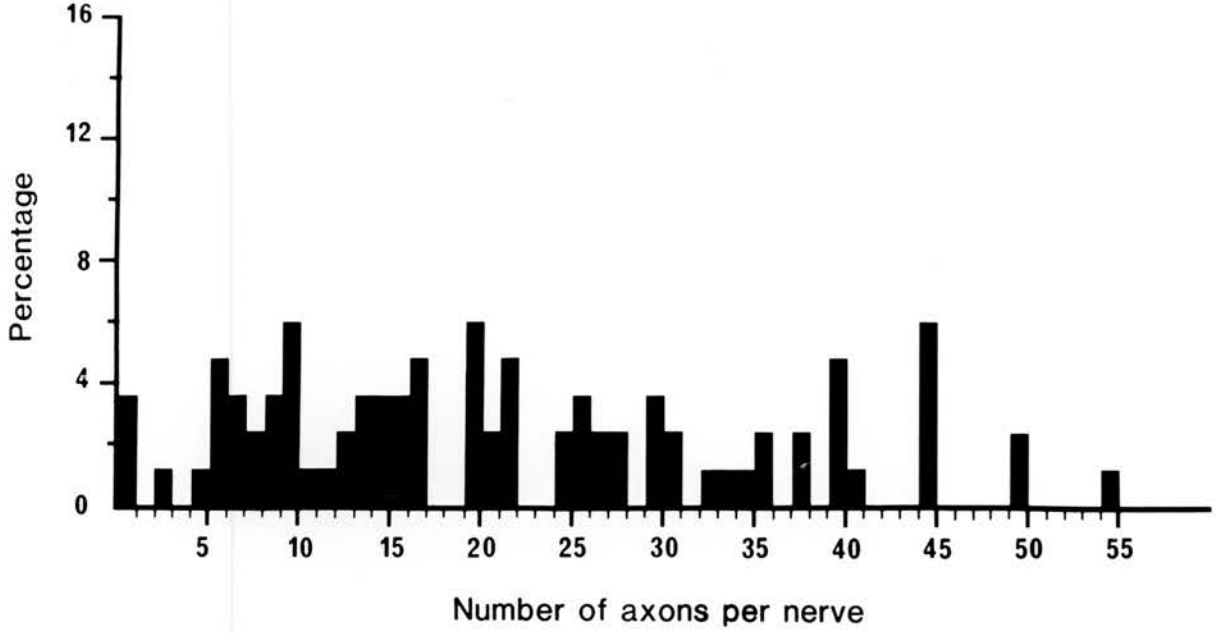
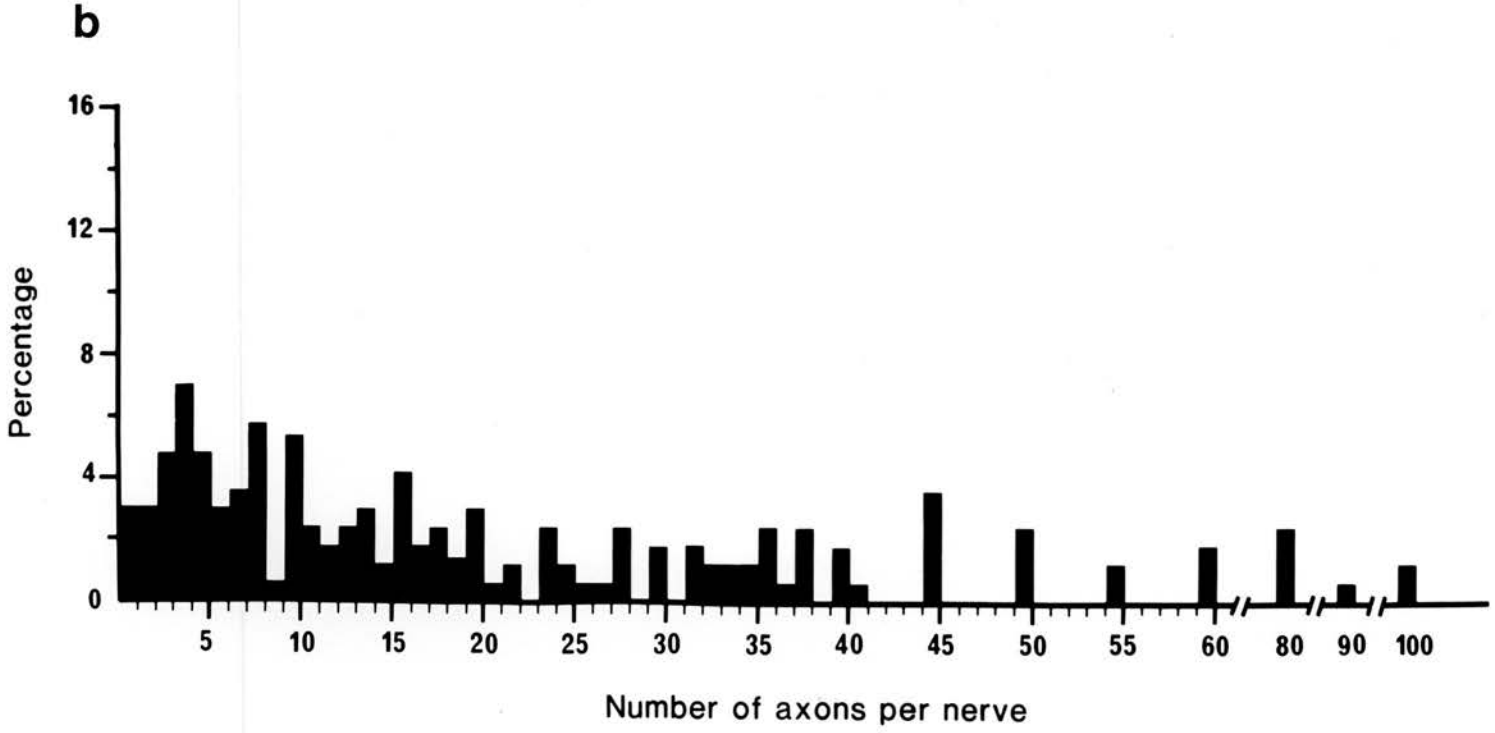

Sc
a1
m
a2

Fig. 43 a, b, c, d, e. Percentage histograms to show the distribution of the number of axon profiles per nerve bundle in the circular muscle layer at the ileo-caeco-rectal junction. a, ileum 5 mm from the junction; b, base of ileal papilla; c, caecum 5 mm from the junction; d, around caecal orifice; and e, rectum 5 mm from the junction.

a


16
12
8
4
0
Percentage
5
10
15
20
25
30
35
40
45
50
55
Number of axons per nerve


b
16
12
8
4
0
Percentage
5
10
15
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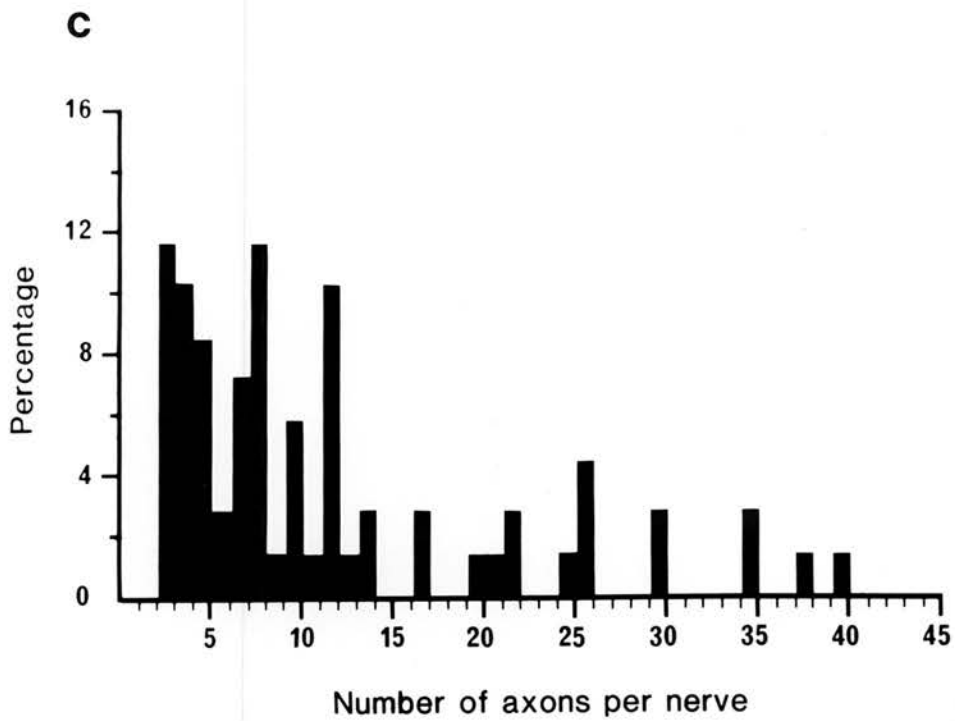
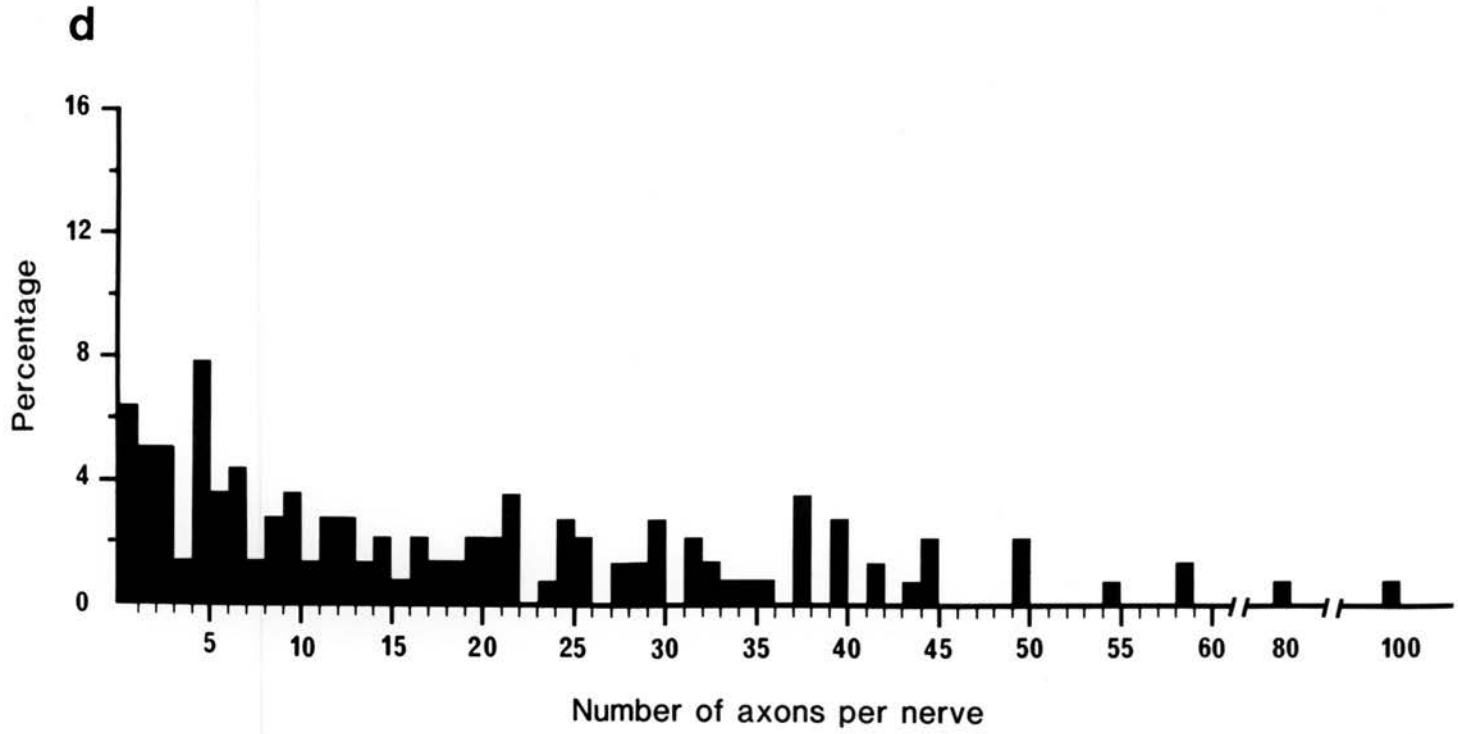
30
35
40
45
50
55
60
80
90
100
Number of axons per nerve


C
16
12
8
4
0
Percentage
5
10
15
20
25
30
35
40
45
Number of axons per nerve


d
16
12
8
4
0
Percentage
5
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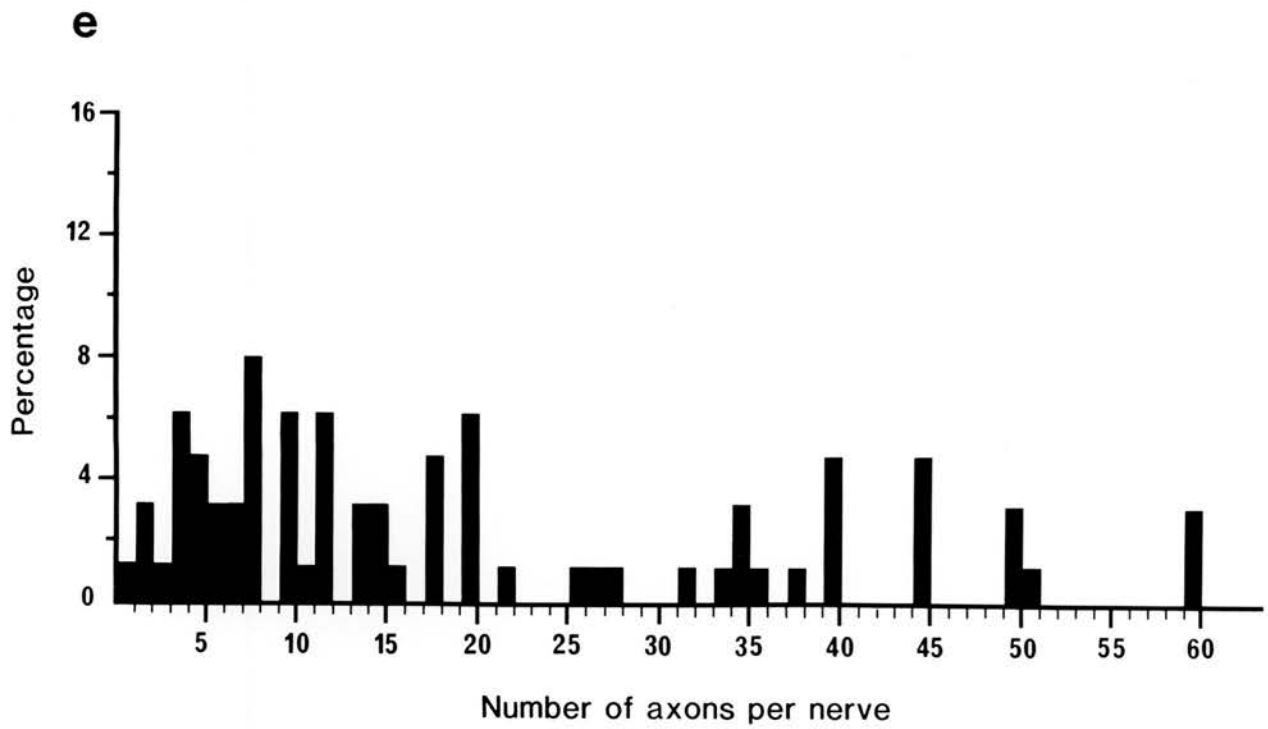
30
35
40
45
50
55
60
80
100
Number of axons per nerve


e
16
12
8
4
0
Percentage
5
10
15
20
25
30
35
40
45
50
55
60
Number of axons per nerve

In transverse sections of the nerve bundles most of the axon profiles (Fig. 45) were oval to round in cross-section. The varicose portions measured up to 1.6 μm in diameter, the intervaricose portions measuring less than 0.3 μm in diameter. In longitudinal sections of the axons the varicose structure became more apparent as the number of the vesicles increased (Fig. 46).

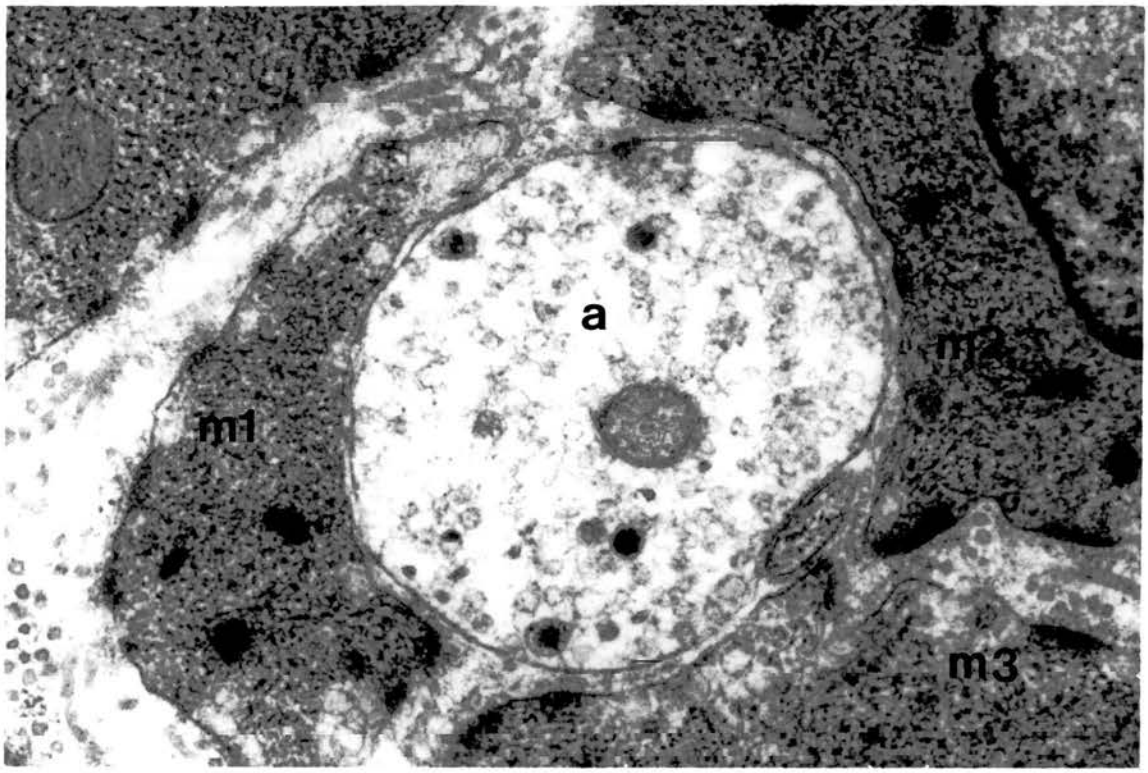
Generally, the axon profiles within the nerve bundles of the circular muscle of all the regions were of two types. Many profiles (Fig. 45) were relatively small, less than 0.3 μm in diameter, and contained neurotubules and mitochondria. Other axon profiles (Fig. 45) were large in diameter, up to 1.6 μm, and their axonal cytoplasm was filled with vesicles of different sizes.

Two types of vesicle within the the varicosities were recognised, agranular and granular. The agranular vesicles appeared to be regular in size and shape. All were round and measured 40-70 nm in diameter (Fig. 47). The granular vesicles were either small (45-75 nm in diameter) or large (80-150 nm in diameter) (Figs. 42, 46, 47). In some, but not all, large granular vesicles the central core was electron-dense and appeared to be separated from the limiting membrane by a clear zone 10-15 nm in diameter (Fig. 47). Elongated or dumb-bell shaped granular vesicles were very rarely seen (Fig. 48).

The axonal enlargements were of three basic varieties according to the type of vesicle which they contained. The first type of varicosity (Figs. 47, 49) contained mainly numerous agranular vesicles and a few large granular vesi-

Fig. 44. Transmission electron micrograph of a transverse section of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction. The axon profile (a) contains many agranular vesicles and a few large granular vesicles and lies close to three muscle cells (m1, m2, m3). X 55000.

Fig. 45. Transmission electron micrograph of a nerve bundle lying between the muscle cells of the circular muscle layer at the base of the ileal papilla. The intervaricose axon profiles (a1) measure less than 0.3 μm in diameter and contain neurotubules. The varicose axon profiles (a2) measure up to 1.6 μm and are filled with vesicles of different sizes. m, muscle cell. X 43000.


a
m1
m2
m3

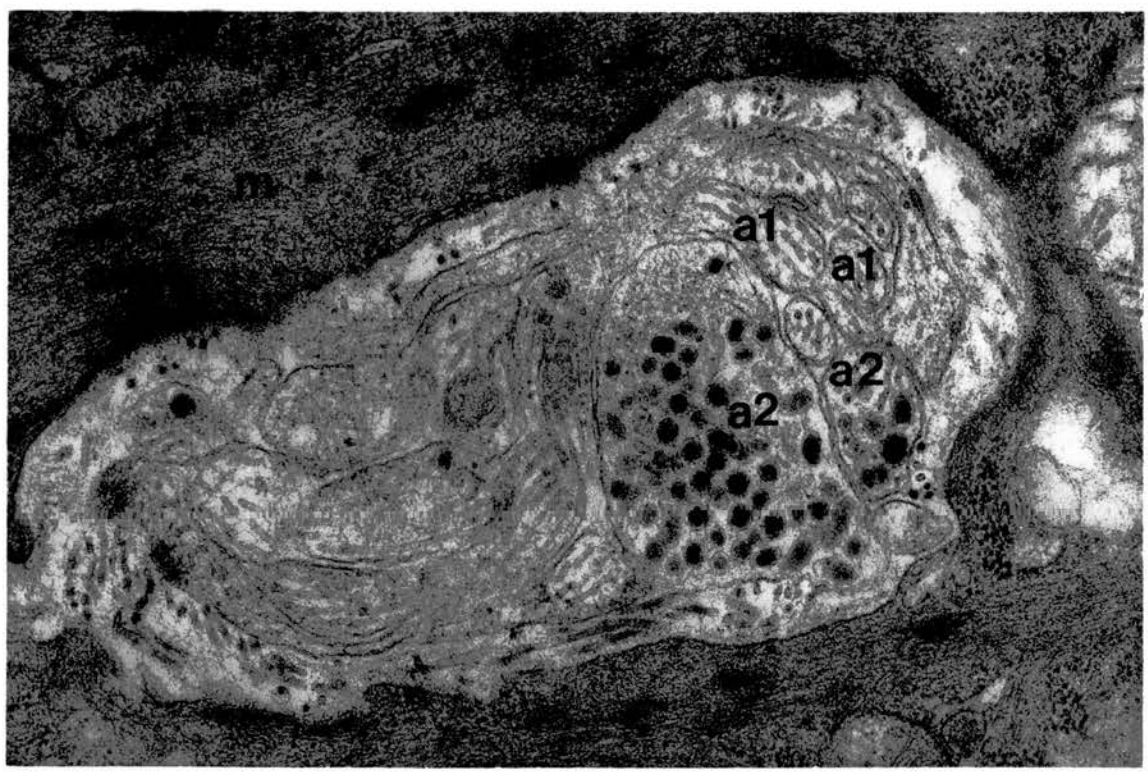

m
a1
a1
a2
a2

Fig. 46. Transmission electron micrograph of a longitudinal section of a nerve bundle lying between the muscle cells of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction. The vesiculated axon profile (a1) contains numerous granular vesicles. The axon profile (a2) contains many agranular vesicles. m, muscle cell. X 40000.

Fig. 47. Transmission electron micrograph of a nerve bundle lying between the muscle cells of the circular muscle layer around the caecal orifice. Type one (v1) and type three (v2) varicosities are present. The varicosity (v1) contains numerous agranular vesicles and a few large granular vesicles. The varicosity (v2) contains many large granular vesicles and many agranular vesicles. The core of some of the vesicles (large arrows) is indistinct. In a few of the granular vesicles the dense core is separated from the limiting membrane by a clear zone (small arrows). m, muscle cell; Sc, Schwann cell process. X 52500.


m
a1
a2

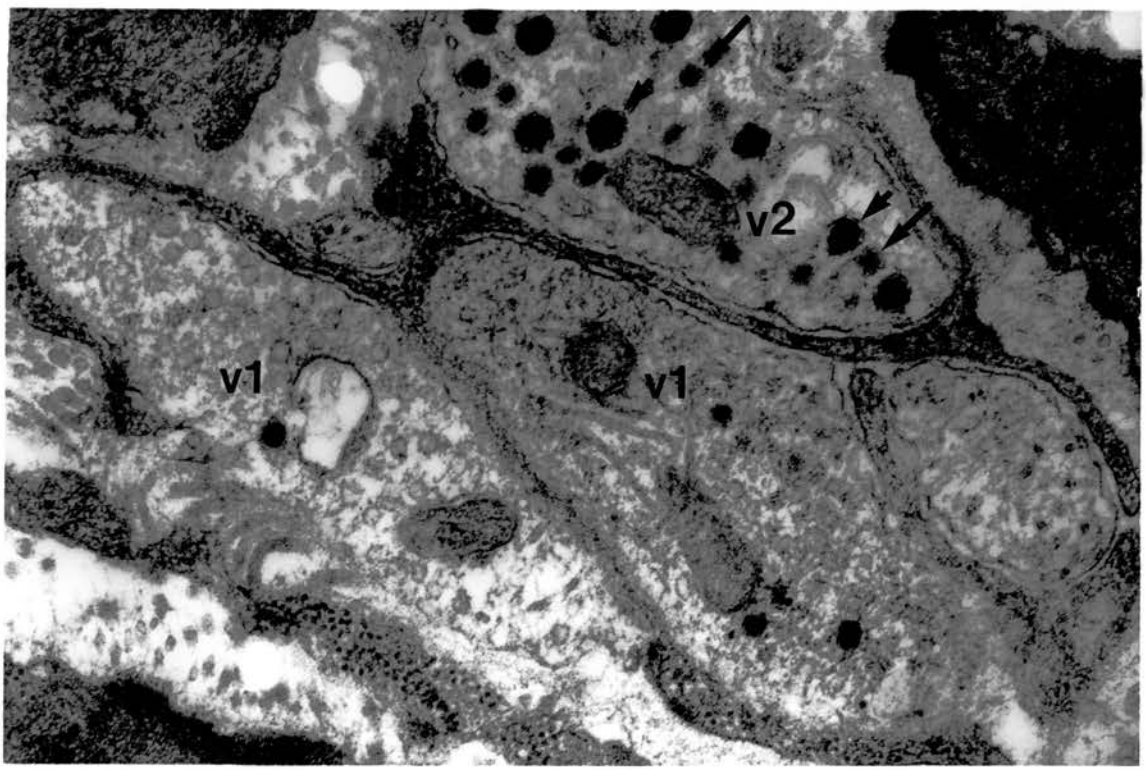
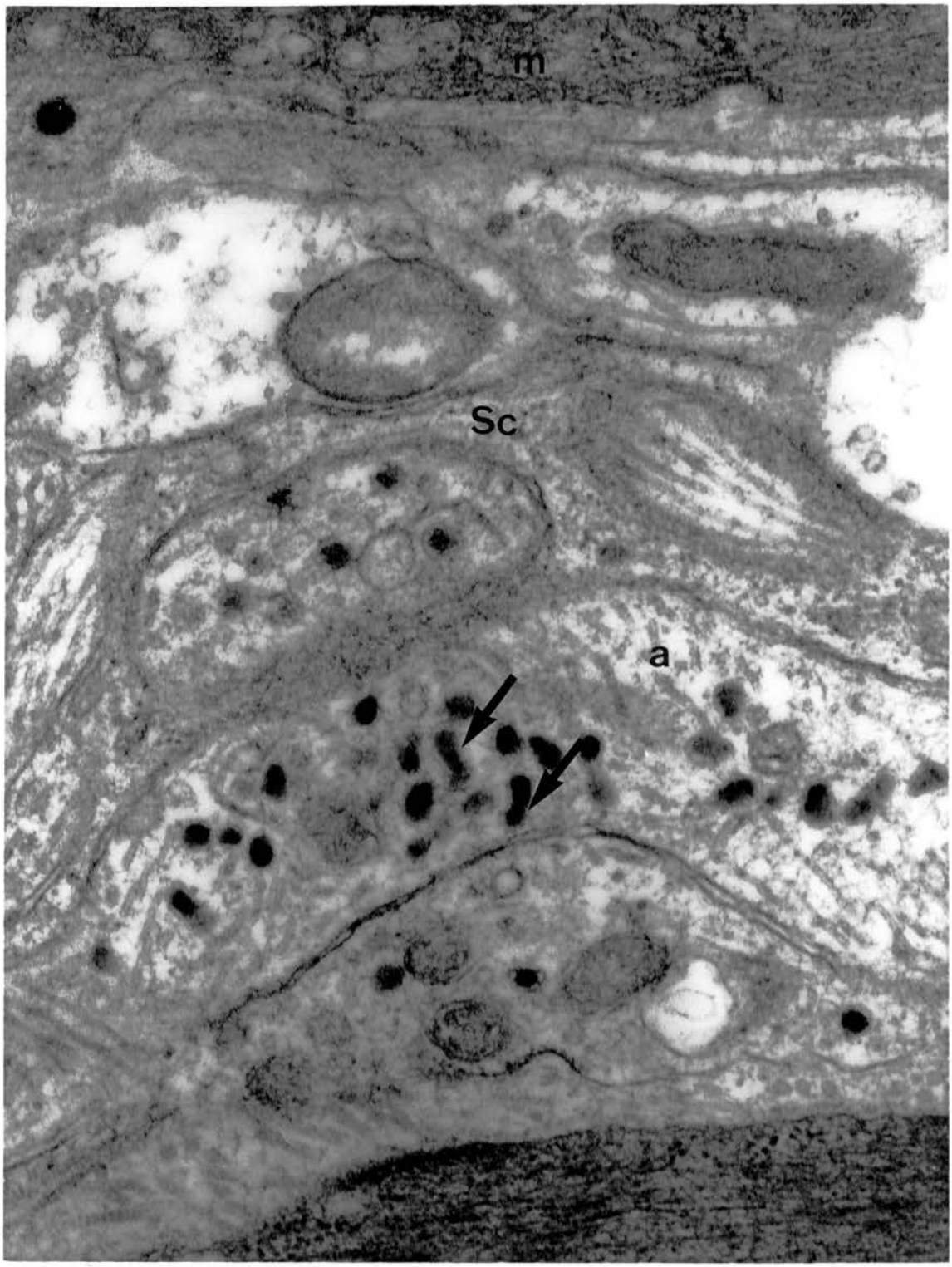

v2
v1
v1

Fig. 48. Transmission electron micrograph of a nerve bundle lying between the muscle cells of the circular muscle layer of the rectum 5 mm from the ileo-caeco-rectal junction. The vesiculated axon profile (a) contains elongated or dumb-bell shaped granular vesicles (arrows). m, muscle cell; Sc, Schwann cell process. X 18000.


m
Sc
a

cles (80-120 nm in diameter). However, varicosities containing only agranular vesicles were occasionally observed. The second type of varicosity (Fig. 49) contained many small granular vesicles, a few large granular vesicles and many agranular vesicles. The third type of varicosity (Figs. 42, 47, 49) contained mainly many large granular vesicles (90-150 nm in diameter) and many agranular vesicles.

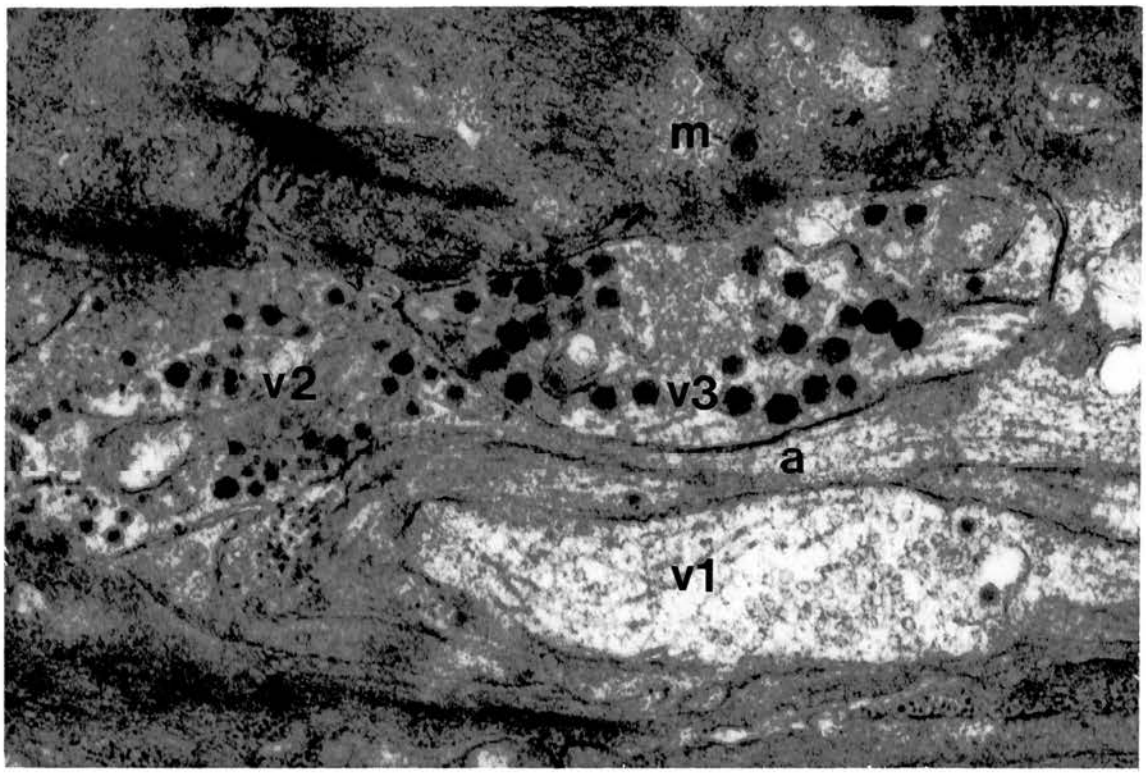
The distance between vesiculated axon profiles and smooth muscle cells ranged from 100 nm to several hundred nanometers although a few junctional gaps between 20 and 40 nm in width were observed in the circular muscle at the base of the ileal papilla. Most of the vesiculated axon profiles appeared to be situated at the surface of the nerve bundles. Partially naked vesiculated axon profiles were seen to lie adjacent to smooth muscle cells, interstitial cells and large areas of intercellular space (Fig. 50).

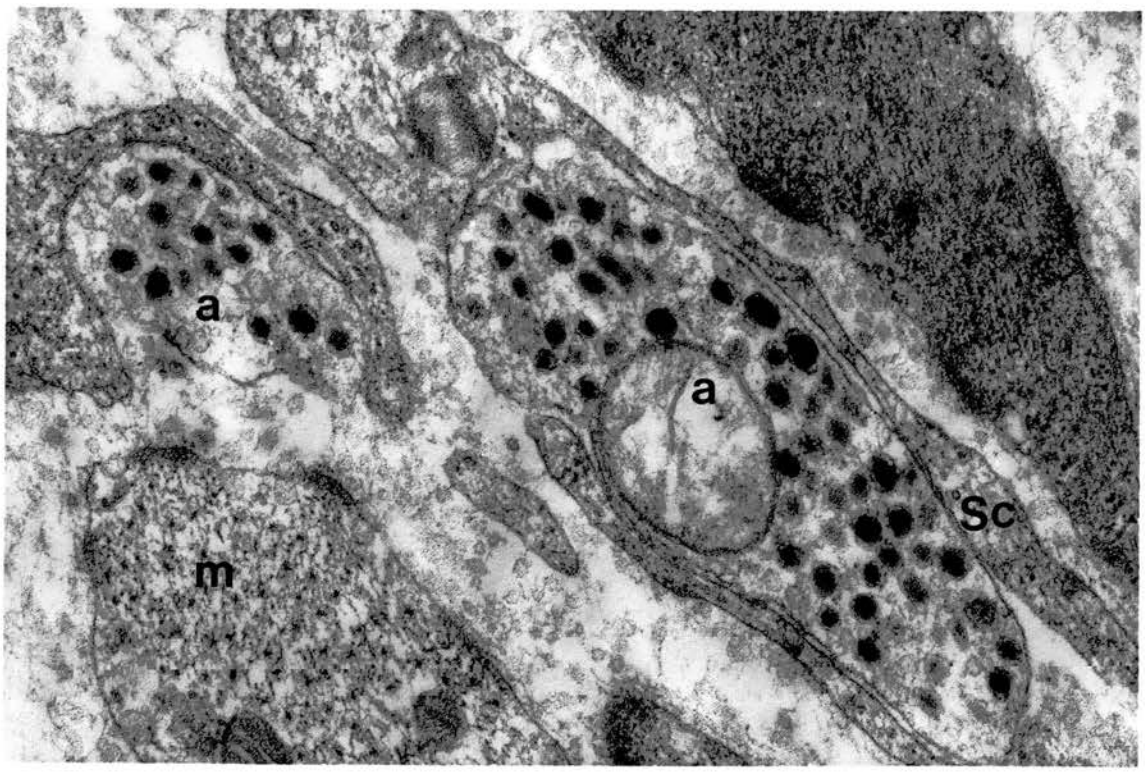
Non-neuronal cells.

The non-neuronal cells associated with the nerve bundles were Schwann cells, interstitial cells and fibroblasts. The last two cells were also found among the muscle cells far away from the nerve bundles.

Fig. 49. Transmission electron micrograph of a nerve bundle lying between the muscle cells of the circular muscle layer at the base of the ileal papilla. Three types of the varicosity can be identified. The varicosity (v1) contains many agranular vesicles and a few large granular vesicles. The varicosity (v2) contains many agranular vesicles, many small granular vesicles, and a few large granular vesicles. The varicosity (v3) contains many agranular vesicles and many large granular vesicles. The non-vesiculated axon profile (a) contains microtubules. m, muscle cell. X 33000.

Fig. 50. Transmission electron micrograph of vesiculated axon profiles in the intercellular space between the muscle cells of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction. Partially naked vesiculated axon profiles (a) lie adjacent to the muscle cells. m, muscle cell; Sc, Schwann cell process. X 44000.


m
v2
v3
a
v1


a
a
Sc
m

(i) Schwann cells.

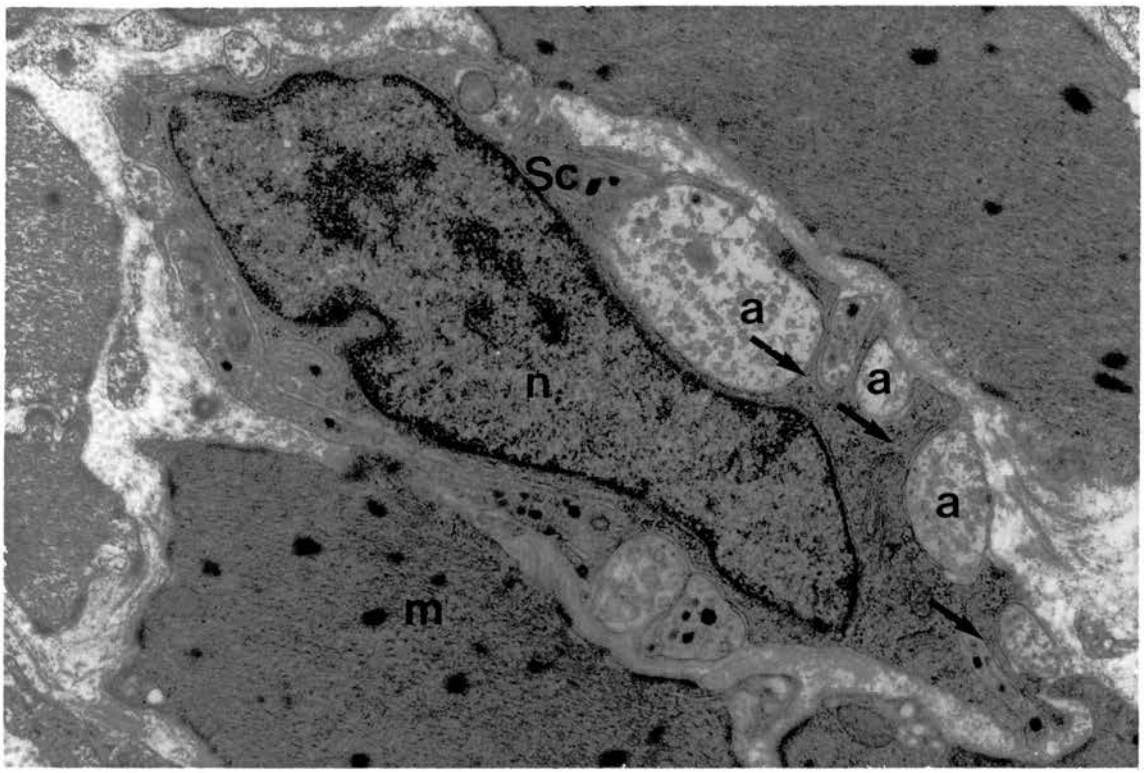
Schwann cells occurred in the nerve bundles and had many processes which enveloped the axons (Fig. 51). Each Schwann cell was completely surrounded by a basal lamina. The cytoplasm of the Schwann cells did not form a complete sheath around each axon and there were small groups of axons in close contact with each other without Schwann cell processes between them. The Schwann cell nucleus was mostly seen in cross-section and was indented and oval or elongated in shape. The heterochromatin was prominent and usually distributed as clumps attached to the inner surface of the nuclear membrane (Fig. 51). The Golgi complex was frequently extensive and lay close to the nucleus. Various cell organelles were found in the cell body including rough endoplasmic reticulum, ribosomes, mitochondria, microtubules, microfilaments and centrioles. The thin Schwann cell processes contained mainly microtubules and microfilaments.

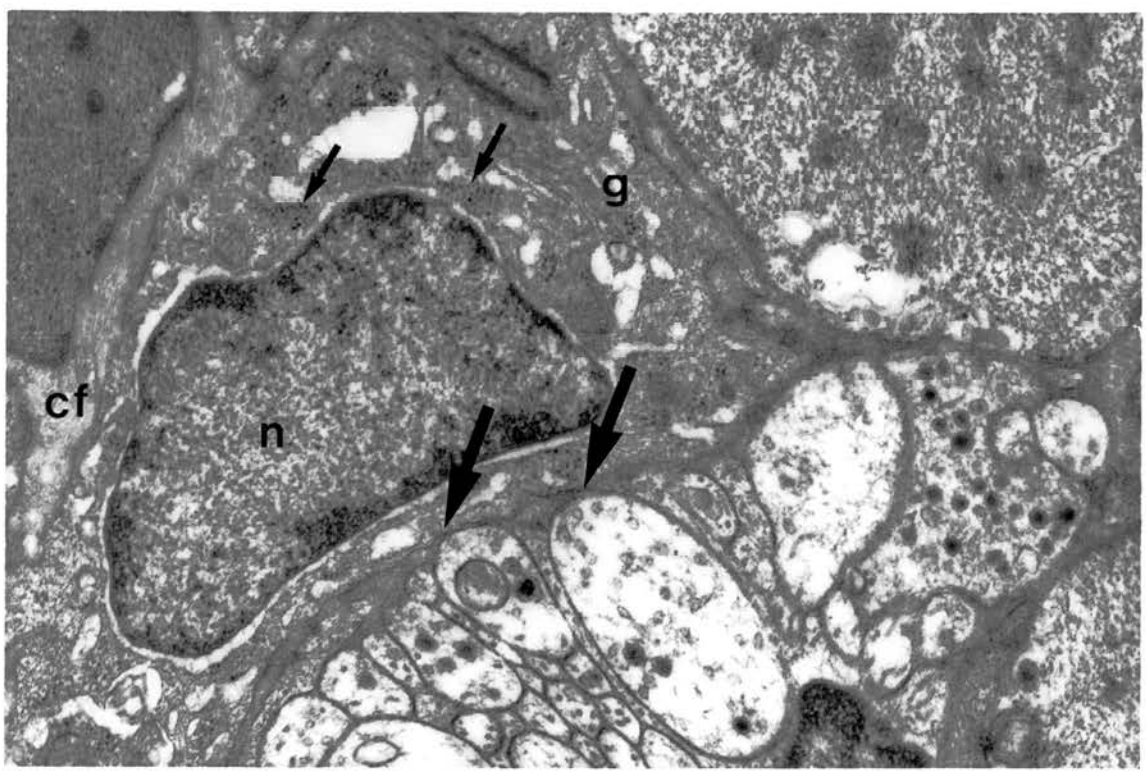
(ii) Interstitial cells.

Interstitial cells (Figs. 52, 55.) were mostly associated with the nerve bundles but were also found among the muscle cells some distance from the nerve bundles. They were observed mainly in the circular muscle layer although

Fig. 51. Transmission electron micrograph of the circular muscle layer at the base of the ileal papilla. A Schwann cell (Sc) is associated with a nerve bundle. The elongated nucleus (n) of the Schwann cell is large and indented and the cytoplasm has many processes (arrows) which envelop the axons (a). m, muscle cell. X 20000.

Fig. 52. Transmission electron micrograph of the circular muscle layer at the caecal orifices showing an interstitial cell which is associated with a bundle of axon profiles (large arrows). The nucleus (n) is large and oval and the heterochromatin is attached to the inner surface of the nuclear membrane. The Golgi complex (g) lies close to the nucleus and the ribosomes (small arrows) are distributed throughout the cytoplasm. cf, collagen fibres. X 30000.


Sc
a
a
a
n
m


g
cf
n

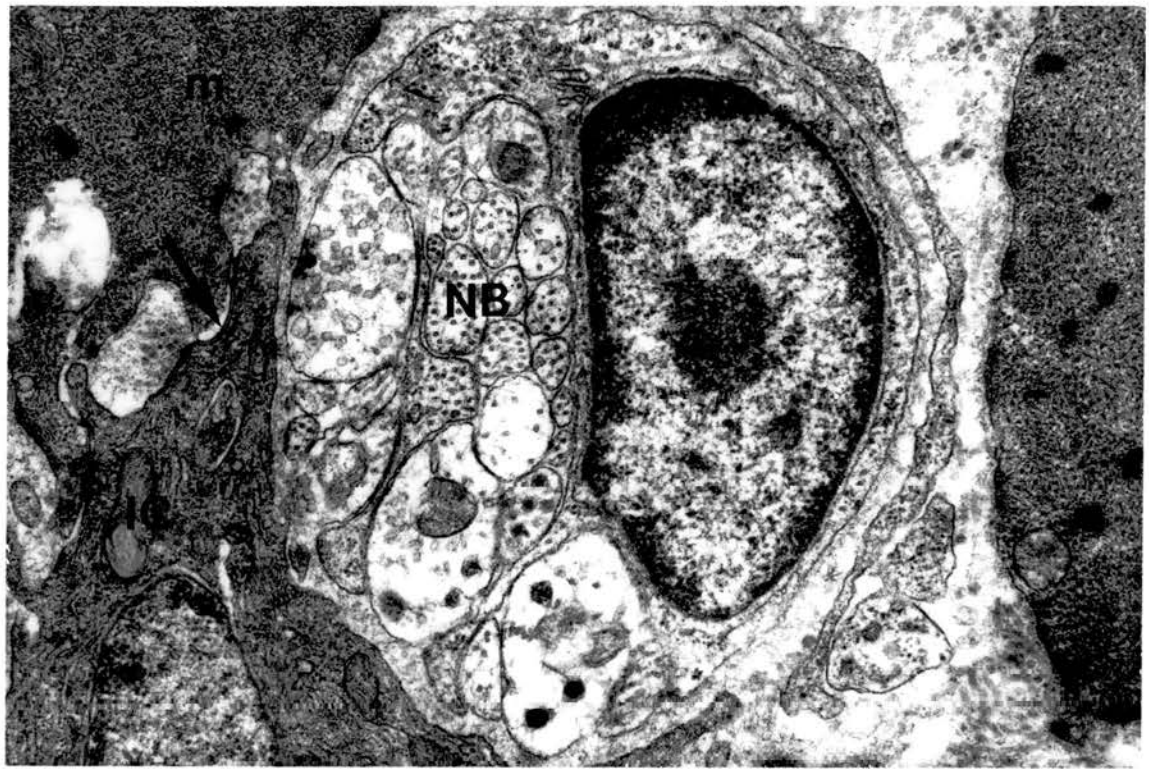
a few were present in the inner and outer longitudinal layers. They were absent in the inner portion of the circular muscle layer at the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction. At this level many large interstitial cells were found closely associated with the large nerve bundles in the space between the inner and outer circular muscle tunics (Fig. 40). They extended along the nerve bundles, branching and giving off many long, thin and irregular processes which extended between the muscle cells (Fig. 57).

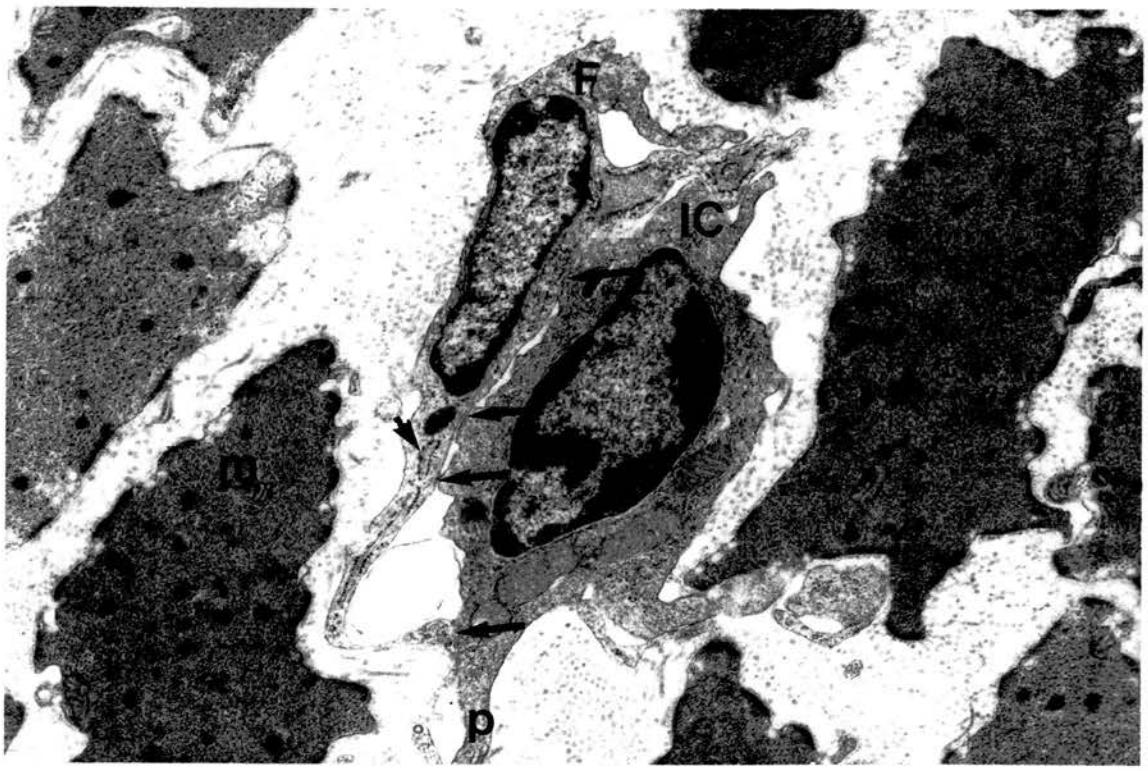
The nucleus of the interstitial cell was large, oval or elongated and had an irregular outline (Fig. 52). The heterochromatin was usually in the form of a wide band attached to the inner surface of the nuclear membrane with occasional clumps being present in the nucleoplasm (Fig. 54). In the cytoplasm there were large, elongated or round mitochondria with transverse cristae and dense intercristal matrix. Sacs and tubules of smooth endoplasmic reticulum, Golgi complex, free ribosomes, occasional lysosome-like bodies, microfilaments, microtubules and vesicles were also observed (Figs. 52, 54). The processes contained free ribosomes, microfilaments and a very few microtubules.

No basal lamina was seen at the surface of the cells. Interstitial cells had a close contact with the nerve bundles where the intercellular gap was reduced to about 20-25 nm. In one muscle cell a finger-like process was observed protruding from the cell towards an interstitial cell to form a gap junction (Fig. 53). Membrane apposition also occurred between the interstitial cells and fibroblasts

Fig. 53. Transmission electron micrograph of the circular muscle layer of the caecum 5 mm from the ileo-caeco-rectal junction. The interstitial cell (IC) forms a gap junction (arrow) with a muscle cell process. m, muscle cell; NB, nerve bundle. X 30000.

Fig. 54. Transmission electron micrograph of the circular muscle layer of the rectum 5 mm from the ileo-caeco-rectal junction. The interstitial cell (IC) in the intercellular space has a large nucleus and many processes (p) which are in close apposition with the body and processes of a fibroblast (long arrows). The fibroblast (F) is a narrow cell with elongated nucleus. The rough endoplasmic reticulum (short arrows) is extensive and continues into the thin cytoplasmic processes. m, muscle cell. X 18000.


m
NB
IC


F
IC
m
p

(Figs. 54, 57), the gap between them being reduced to about 10-20 nm.

(iii) Fibroblasts.

Fibroblasts (Figs. 54, 55, 57) occurred among the collagen fibres, between muscle cells and close to nerve bundles. They were observed mainly in the circular muscle layer, but a few were also seen in the inner and outer longitudinal muscle layers. They were never observed between the muscle cells of the inner portion of the circular muscle layer at the terminal part of the ileum 5 mm from the ileo-caeco-rectal junction.

Fibroblasts were narrow with elongated nuclei and thin, long branching cytoplasmic processes extending between the muscle cells (Figs. 54, 57). Within the nucleus was a thin band of heterochromatin attached to the inner surface of the nuclear membrane. The cytoplasm of the cell body contained large, swollen and extensive rough endoplasmic reticulum. Free ribosomes, a few mitochondria, Golgi areas, lysosome-like structures and occasional vesicles were also observed (Fig. 55). The cytoplasmic processes contained rough endoplasmic reticulum, free ribosomes and filaments (Figs. 55, 56, 57). A basal lamina was never seen around these cells. Fibroblasts came in close contact with nerve bundles the gap between them being reduced to about 10-20 nm. There was also contact with muscle cells the gap being reduced to 20-30 nm.

Fig. 55. Transmission electron micrograph of the circular muscle layer at the caecal orifice. The fibroblast cytoplasm contains an extensive rough endoplasmic reticulum (rer), Golgi complex (g), and vesicles (arrows). ic, intercellular space; m, muscle cell; n, nucleus; p, cytoplasmic process. X 25000.

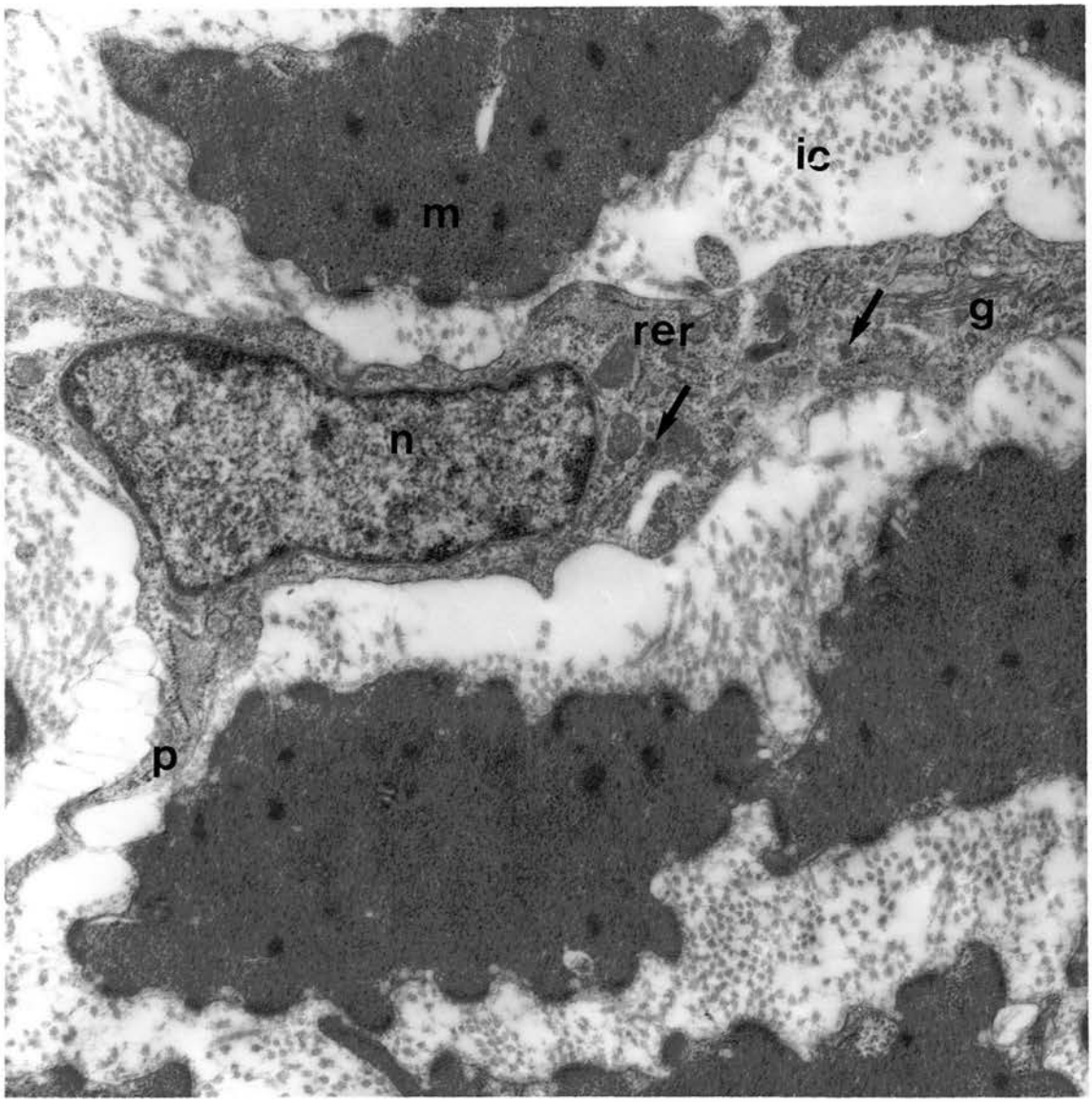
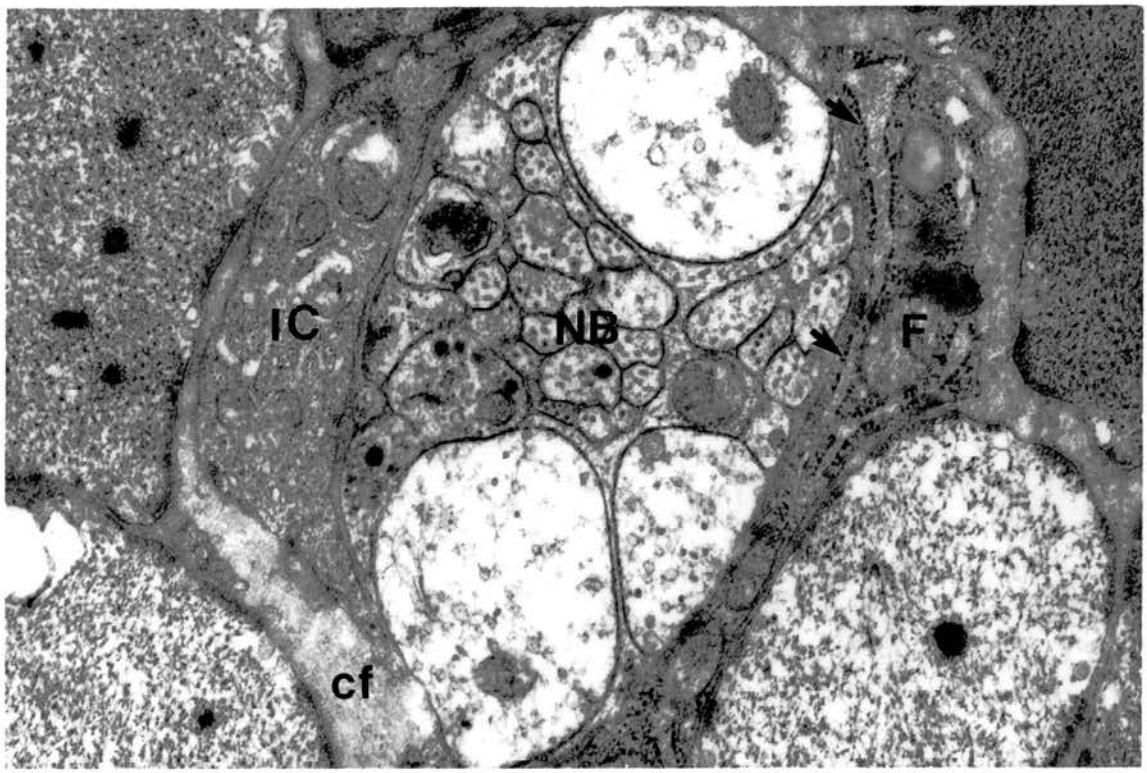

ic
m
rer
g
n
p

Fig. 56. Transmission electron micrograph of the circular muscle layer at the base of the ileal papilla. The interstitial cell (IC) and the fibroblast (F) are associated with the nerve bundle (NB). Note the extensive endoplasmic reticulum (arrows) in the cytoplasm of the fibroblast. cf, collagen fibres; m, muscle cell. X 30000.

Fig. 57. Transmission electron micrograph of the circular muscle at the base of the ileal papilla. The very long processes of the interstitial cell (ICP) and fibroblast (FP) extend between the muscle cells and are in close apposition to each other at many points (arrows). cf, collagen fibres; m, muscle cell. X 30000.


IC
NB
F
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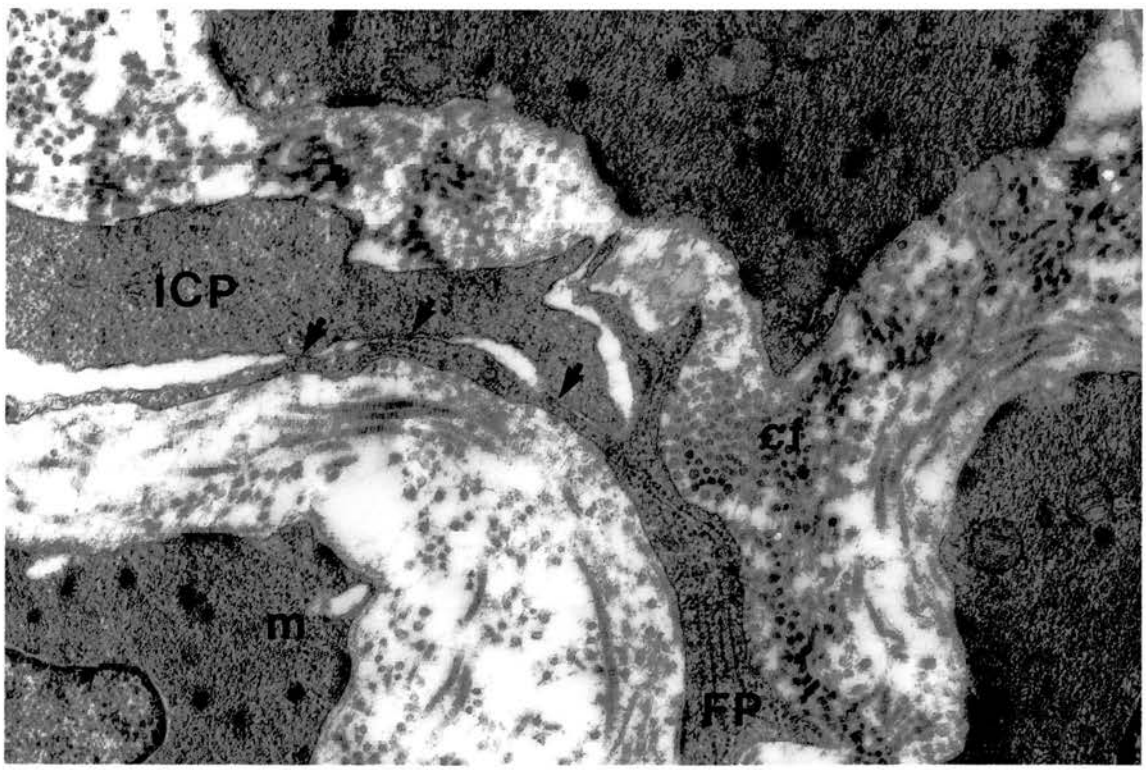

ICP
cf
m
FP

(7) Quantitative Observations on the Innervation in the Region of the Ileo-Caeco-Rectal Junction.

The quantitative study of the innervation of the circular muscle of the ileo-caeco-rectal region (Table 7) showed that the innervation of the muscular rings at the base of the ileal papilla (31 nerve bundles/ 1000 muscle fibres) and around the caecal orifice (26 nerve bundles/ 1062 muscle fibres) was more dense than the innervation of the non-thickened circular muscle 5 mm from the junction in the ileum (15nerve bundles/ 1100 muscle fibres), caecum (14 nerve bundles/ 1120 muscle fibres) and rectum (13 nerve bundles/ 1022 muscle fibres).

The total number of axon profiles counted per number of circular muscle cell profiles was 472 axons/ 1000 muscle cells at the base of the ileal papilla and 352 axons/ 1062 muscle cells around the caecal orifice. 5 mm from the junction the number of axon profiles per number of circular muscle cell profiles was 295 axons/ 1100 muscle cells in the ileum, 197 axons/ 1120 muscle cells in the caecum and 246 axons/ 1022 muscle cells in the rectum.

The number of vesiculated axon profiles and their percentage of the total number of axon profiles was greater in the circular muscle at the base of the

Table 7

Distribution of nerve bundles and axon profiles in the circular muscle of the intestine in the region of the ileo-caeco-rectal junction of ten birds.

Region	No. of circular muscle fibres	No. of nerve bundles	Total no. of axon profiles	No. of non-vesiculated axon profiles	No. of vesiculated axon profiles
Ileum 5 mm from junction	1100	15	295 (100%)	248 (84%)	47 (16%)
Base of ileal papilla	1000	31	472 (100%)	329 (69.7%)	143 (30.3%)
Rectum 5 mm from junction	1022	13	246 (100%)	205 (83.4%)	41 (16.6%)
Caecal orifice	1062	26	352 (100%)	241 (68.5%)	111 (31.5%)
Caecum 5 mm from junction	1120	14	197 (100%)	166 (84.2%)	31 (15.8%)

ileal papilla (143, 30.3%) and around the caecal orifice (111, 31.5%) than 5 mm from the junction in the ileum(47, 16%), caecum (31, 15.8%) and rectum (41, 16.6%).

Statistical analysis by the " t test " showed that the innervation was significantly denser and contained more vesiculated axon profiles ($P \leq 0.01$) in the thickened rings at the base of the ileal papilla and around the caecal orifices than in the ileum, caecum and rectum 5 mm from the junction (Table 8).

B. RECTO-COPRODEAL JUNCTION.

(1) Gross Observations.

In the distended gut of the female duck the terminal part of the rectum was tubular in shape and 5 mm from the junction its diameter was about 7.5-9.6 mm and this decreased slightly at the recto-coprodeal junction where it was 5.4-6.5 mm. The lumen of the coprodeum was considerably wider than that of the rectum being about 10.5-12.8 mm in diameter 5 mm from the junction. Whilst a true macroscopic fold was not observed at the recto-coprodeal junction the transition of the mucosa from a light brown colour in the rectum to white in the coprodeum was marked by an irregular line. 1-2 mm caudal to the junction there was an abrupt change in the gross appearance of the mucosa from a velvet

Table 8

Distribution of axon profiles and vesiculated axon profiles in the circular muscle of the intestine in the region of the ileo-caeco-rectal junction of ten birds.

Region	Total no. of axon profiles	Statistical significance (t test)	No. of vesiculated axon profiles	Statistical significance (t test)
Ileum 5 mm from junction	295 (100%)		47 (16%)	
		t=10.6 (p<0.01)		t=12.3 (p<0.01)
Base of ileal papilla	472 (100%)		143 (30.3%)	
		t=11.7 (p<0.01)		t=13.1 (p<0.01)
Rectum 5 mm from junction	246 (100%)		41 (16.6%)	
		t=5.07 (p<0.01)		t=10.2 (p<0.01)
Caecal orifice	352 (100%)		111 (31.5%)	
		t=8.3 (p<0.01)		t=13.1 (p<0.01)
Caecum 5 mm from junction	197 (100%)		31 (15.8%)	

surface of villi to a smooth shiny surface (Fig. 58).

(2) Histological Study of the Musculature of the Recto-Coprodeal Junction.

The musculature in the wall of the rectum and coprodeum consisted of the muscularis mucosae and the muscle tunic.

(a) Muscularis mucosae.

The muscularis mucosae of the recto-coprodeal junction consisted of longitudinally-orientated loosely packed muscle fibres. The muscularis mucosae of the rectum measured about 50-70 μm in thickness 5 mm from the recto-coprodeal junction, 80-120 μm at the junction and 25-40 μm in the coprodeum.

(b) Muscle tunic.

The muscle tunic at the recto-coprodeal junction consisted of a thick inner circular layer and a thin outer longitudinal layer (Fig. 59).

Fig. 58. At the recto-coprodeal junction the transition of the mucosa from a light-brown velvet surface in the rectum (R) to a white smooth shiny surface in the coprodeum (C) is marked by an irregular line (arrows). Scale, 2 mm

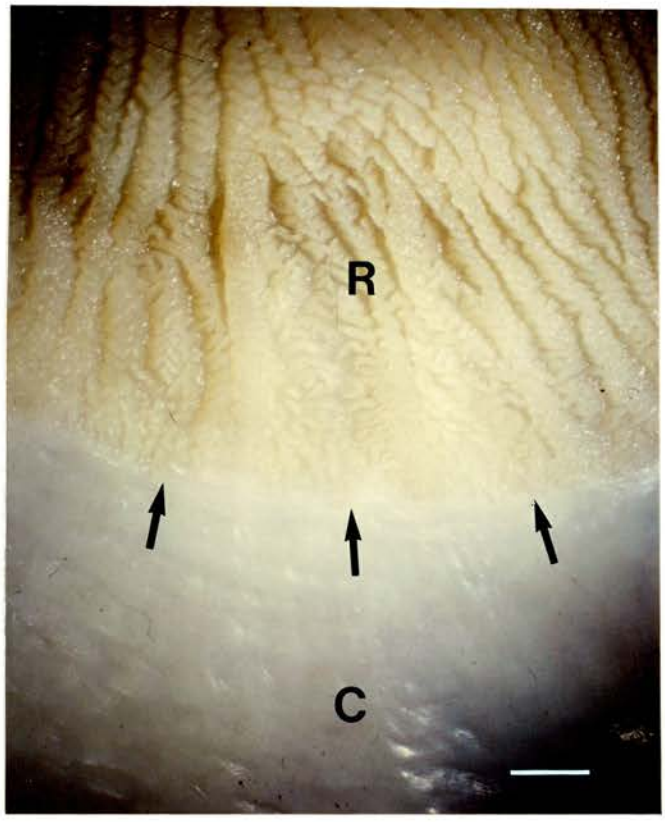
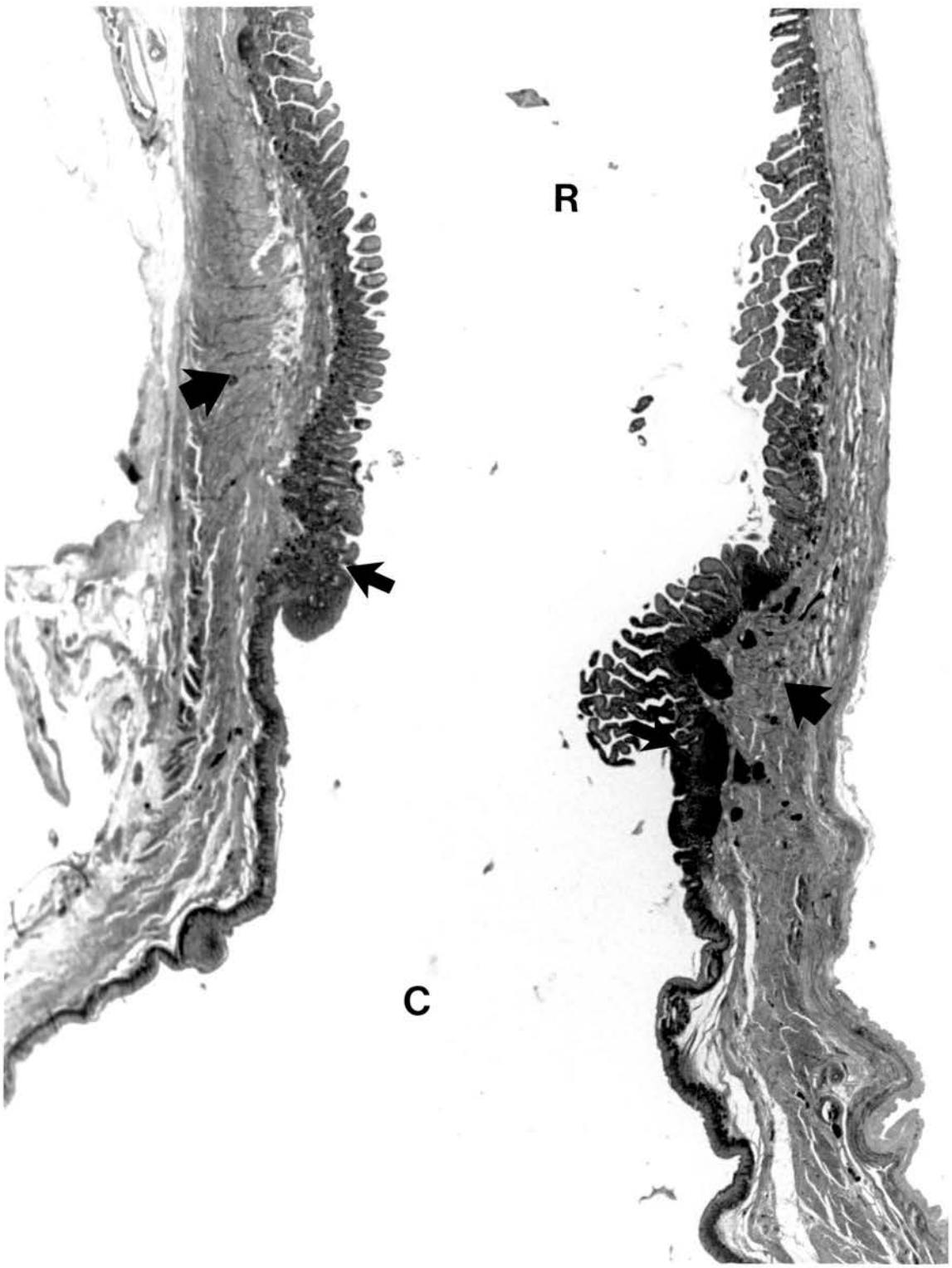

R
C

Fig. 59. Light micrograph of a longitudinal section of the recto-coprodeal junction. The circular muscle layer of the rectum 1-2 mm cranial to the region of the junction is slightly thickened forming an obliquely orientated ring (large arrows). The junction between the rectum and the coprodeum is indicated by small arrows. C, coprodeum; R, rectum. X 10.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(i) Longitudinal muscle layer.

The longitudinal muscle layer at the recto-coprodeal junction consisted of irregularly arranged muscle fibres and was separated from the circular muscle layer by a thin layer of connective tissue.

At the rectum 5 mm from the junction and at the recto- coprodeal junction the layer was about 150-195 μm thick. In the coprodeum 5 mm from the junction the layer measured about 82-112 μm in thickness. Thus no circumscribed thickening of the muscle layer was observed at the junction.

(ii) Circular muscle layer.

The circular muscle of the rectum 5 mm from the junction with the coprodeum was composed of closely packed muscle fibres and was about 375-450 μm thick. It was separated from the muscularis mucosae by a thin layer of connective tissue. 1-2 mm cranial to the recto-coprodeal junction the circular muscle of the rectum (Fig. 59) was thickened forming an obliquely orientated ring which measured about 550-625 μm in thickness, the dorsal part of the thickened muscle lying cranial to the ventral part. The circular muscle of the coprodeum was about 310-395 μm thick.

(3) Ultrastructure of the Muscle Cells in the Region of the Recto-Coprodeal Junction.

The muscularis mucosae consisted of 6-10 rows of muscle cells and was separated from the circular layer by a wide band of connective tissue containing few nerve bundles and blood vessels. The muscle fibres were grouped into small, distinct bundles, the diameter of the fibres in the nuclear portion being about 2.8-3.2 μm.

The circular muscle layer was single and formed the bulk of the muscle tunic. The muscle fibres were grouped into large bundles and had a diameter at their nuclear portion of 3.6-4.2 μm. The layer was separated from the longitudinal muscle layer by a wide sheet of connective tissue containing nerve bundles and blood vessels. The muscle cells of the circular layer were not observed to be in contact with those of the muscularis mucosae or the longitudinal muscle layer.

The longitudinal muscle layer formed the outermost layer of the muscle tunic and was 7-10 cells thick. The muscle fibres of this layer were grouped into distinct, small bundles and had a diameter at their nuclear portion of 3-3.3 μm.

The ultrastructure of the muscle cells and their junctions was the same as described for the muscle cells in the ileo-caeco-rectal junction on pages 52 and 55.

(4) Quantitative Observations on the Musculature in the Region of the Recto-Coprodeal Junction.

(a) Measurements of muscle cell length.

The measurement of muscle cell length (Tables 9, 10) was based on the formula used by Gabella (1976) and has been described on page 57.

The lengths of the muscle cells ranged from 92-105 μm in the muscularis mucosae, 97-139 μm in the longitudinal layer and 175-282 μm in the circular layer. In the circular layer the longest muscle cells occurred directly opposite the recto-coprodeal junction. The shortest cells in all three layers were present in the middle part of the rectum.

In the circular muscle layer there were apparent differences in the length of the muscle cells between those at the recto-coprodeal junction (where they were the longest) and those in the rectum and coprodeum 5 mm from the junction. In all muscle layers apparent differences were also found between the length of the muscle cells in the middle part of the rectum (where they were the shortest) and those in the rectum 5 mm from the junction. However, the number of specimens was too small to establish statistically significant differences (Table 11).

Table 9

The length of the muscle cells nuclei (μm) and the percentage of the nucleated muscle cell profiles at the recto-coprodeal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer.

Region		Middle portion of the rectum		Rectum 5mm from junction		Recto-coprodeal junction		Coprodeum 5 mm from junction	
		Nucleus length (μm)	% of nucleated muscle cell profiles	Nucleus length (μm)	% of nucleated muscle cell profiles	Nucleus length (μm)	% of nucleated muscle cell profiles	Nucleus length (μm)	% of nucleated muscle cell profiles
MM	1	16.04	12.78	13.68	11.04	15.12	16.07	13.00	12.20
	2	12.28	13.28	14.75	16.80	14.09	11.08	11.46	11.62
	3	8.92	15.46	8.97	8.71	12.35	14.00	10.90	13.69
CM	1	15.20	9.66	17.60	7.97	20.18	9.30	16.10	6.55
	2	14.30	7.91	16.21	7.09	18.36	4.22	14.82	7.20
	3	14.45	7.76	15.95	6.42	16.24	8.42	15.27	6.70
LM	1	13.76	13.46	15.72	14.28	15.20	13.27	12.10	8.25
	2	11.56	11.30	14.82	9.61	11.50	8.55	11.63	9.76
	3	10.80	12.50	14.25	11.20	13.46	9.57	10.62	7.05

Table 10

Length of the muscle cell (μm) at the recto-coprodeal region in three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer.

Region		Middle portion of the rectum	Rectum 5mm from junction	Recto-coprodeal junction	Coprodeum 5 mm from junction
	1	125.51	123.91	94.09	106.56
MM	2	92.47	87.80	127.17	98.62
	3	57.70	102.98	88.21	79.62
Mean		91.89	104.90	103.16	94.93
S.D.		± 33.91	± 18.13	± 21.00	± 13.84
	1	157.35	220.83	216.99	245.80
CM	2	180.78	228.63	435.07	205.83
	3	187.37	248.44	192.87	227.91
Mean		175.17	232.63	281.64	226.51
S.D.		± 15.78	± 14.23	± 133.42	± 20.02
	1	102.23	110.08	114.54	146.67
LM	2	102.30	154.21	134.50	119.16
	3	86.40	127.23	140.65	150.64
Mean		96.98	130.51	129.90	138.82
S.D.		± 9.16	± 22.25	± 13.65	± 17.14

Table 11

The mean of the muscle cell length (μm) at the recto-coprodeal junction of three birds compared with the middle portion of the rectum and the rectum and coprodeum 5mm from the junction. MM, muscularis mucosae; CM, circular muscle; LM, longitudinal muscle.

Region	MM	Statistical significance (t-test)	CM	Statistical significance (t-test)	LM	Statistical significance (t-test)
Middle portion of the rectum	91.89	t=0.59 not significant	175.17	t=4.68 (p<0.01)	96.98	t=2.41 not significant
Rectum 5 mm from junction	104.90	t=0.11 not significant	232.63	t=0.63 not significant	130.51	t=0.40 not significant
Recto-coprodeal junction	103.16	t=0.56 not significant	281.64	t=0.71 not significant	129.90	t=0.70 not significant
Coprodeum 5 mm from junction	94.93		226.51		138.82	

(b) Measurements of muscle cell volume.

The measurement of muscle cell volume (Tables 12, 13) was based on the formula used by Gabella (1976) and has been described on page 58.

The volume of the muscle cells in the muscularis mucosae ranged from 567-694 μm^3. The largest volume of the muscle cells (1435-1979 μm^3), occurred in the circular layer, the volume at the recto-coprodeal junction being larger than in the other three regions. The muscle cells of the longitudinal layer had a volume ranging from 718-811 μm^3.

The volume of the muscle cells in the circular layer was greatest at the recto-coprodeal junction and was apparently larger here than in the muscle cells in the rectum and coprodeum 5mm from the junction. In all three muscle layers an apparent difference was also found between the volume of the muscle cells in the middle part of the rectum (where they were the smallest) and those in the rectum 5 mm from the junction. However, the number of specimens was too small to establish statistically significant differences (Table 14).

Table 12

The total surface area (μm^2) and the total number of nucleated muscle muscle cell profiles at the recto-coprodeal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer.

Region		Middle portion of the rectum		Rectum 5mm from junction		Recto-coprodeal junction		Coprodeum 5 mm from junction	
		Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles	Total surface area (μm^2)	Total no. of nucleated muscle cell profiles
		1603.72	57	1776.39	38	1721.46	39	1513.74	21
MM	2	1743.12	36	2065.34	35	2304.30	47	1792.44	26
	3	2207.50	30	2500.06	40	2048.66	65	1941.60	30
	1	4410.54	46	1964.37	25	1867.33	20	2117.96	25
CM	2	1817.04	19	2938.47	29	3008.20	30	2701.79	31
	3	3361.44	33	3515.47	32	2861.19	21	2397.99	33
	1	1208.31	21	1119.83	26	1238.98	28	2023.26	30
LM	2	1552.73	26	2115.68	42	2155.97	30	2980.51	46
	3	1715.27	25	2126.18	30	1813.64	23	1866.59	34

Table 13

Volume of the muscle cell (μm^3) at the recto-coprodeal region of three birds. MM, muscularis mucosae; CM, circular muscle layer; LM, longitudinal muscle layer.

Region		Middle portion of the rectum	Rectum 5mm from junction	Recto-coprodeal junction	Coprodeum 5 mm from junction
	1	451.29	639.50	667.40	578.78
MM	2	594.60	870.39	690.80	790.05
	3	656.36	560.64	722.88	705.45
Mean		567.42	690.18	693.69	691.43
S.D.		± 105.20	±160.97	±27.85	±106.33
	1	1457.39	1382.92	1884.14	1363.97
CM	2	1367.56	1642.50	1841.01	1291.63
	3	1481.07	1752.24	2212.65	1109.61
Mean		1435.34	1592.55	1979.27	1255.07
S.D.		± 59.88	±189.66	±203.26	±131.06
	1	791.73	677.07	672.89	816.05
LM	2	690.37	746.53	826.45	753.55
	3	741.00	1009.93	916.24	583.03
Mean		741.03	811.18	805.19	717.54
S.D.		± 50.68	±175.59	±123.06	±120.61

Table 14

The mean of the muscle cell volume (μm^3) at the recto-coprodeal junction of three birds compared with the middle portion of the rectum and the rectum and coprodeum 5 mm from the junction. MM, muscularis mucosae; CM, circular muscle; LM, longitudinal muscle.

Region	MM	Statistical significance (t-test)	CM	Statistical significance (t-test)	LM	Statistical significance (t-test)
Middle portion of the rectum	567.42	t=1.11 not significant	1435.34	t=1.37 not significant	741.03	t=0.66 not significant
Rectum 5 mm from junction	690.17	t=0.37 not significant	1592.55	t=2.41 not significant	811.17	t=0.48 not significant
Recto-coprodeal junction	693.69	t=0.35 not significant	1979.27	t=5.19 (p<0.01)	805.19	t=0.88 not significant
Coprodeum 5 mm from junction	691.43		1255.07		717.54	

(5) Ultrastructure of the Nerve Bundles in the Region of the Recto-Coprodeal Junction.

(a) Nerve bundles.

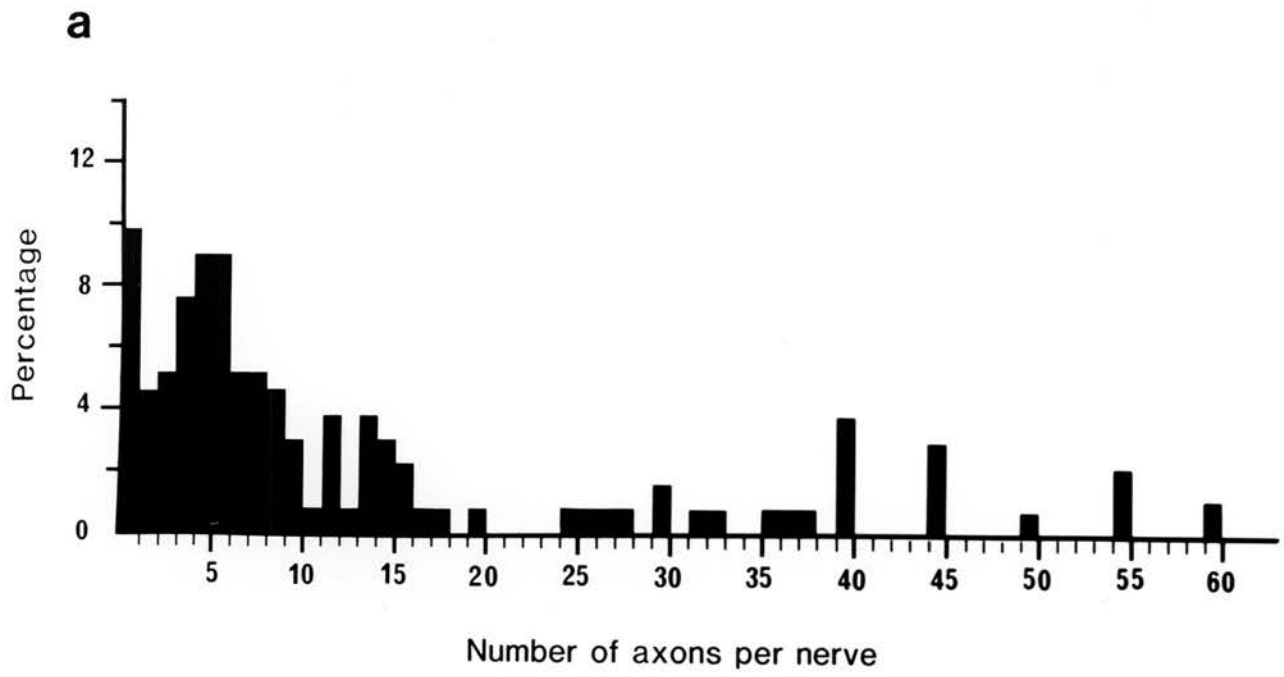
Nerve bundles were present in all the muscle layers. Whilst they were distributed throughout the circular layer, they were rare in both the inner and outer longitudinal layers. The nerve bundles in the circular muscle layer of the middle part of the rectum, the rectum and coprodeum 5 mm from the junction, and the recto-coprodeal junction consisted of 1-150 axons (Fig. 60 a-d), the majority of nerve bundles containing 2-60 axons. Large-sized nerve bundles with 90-150 axons were only observed at the recto-coprodeal junction where they were restricted to the circular layer (Fig. 60 c).

The type of the vesicles and the axonal enlargements was the same as described on page 61 for the ileo-caeco-rectal junction.

(b) Non-neuronal cells.

The non-neuronal cells associated with the nerve bundles in the three muscle layers in the middle part of the rectum, in the rectum and coprodeum 5 mm from the junction, and in the recto-coprodeal junction were Schwann cells, interstitial

Fig. 60 a, b, c, d. Percentage histograms to show the distribution of the number of axon profiles per nerve bundle in the circular muscle layer at the recto-coprodeal junction. a, the middle portion of the rectum; b, the rectum 5 mm from the junction; c, the recto-coprodeal junction; and d, the coprodeum 5 mm from the junction.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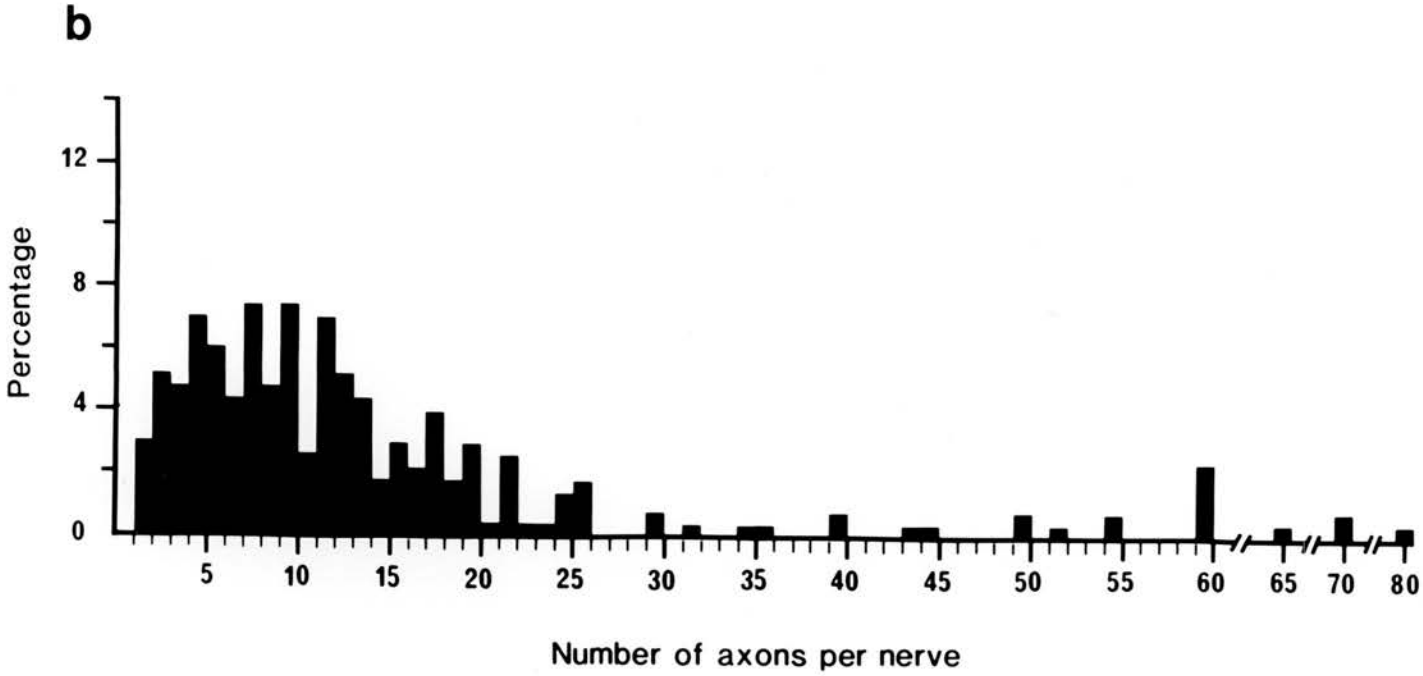
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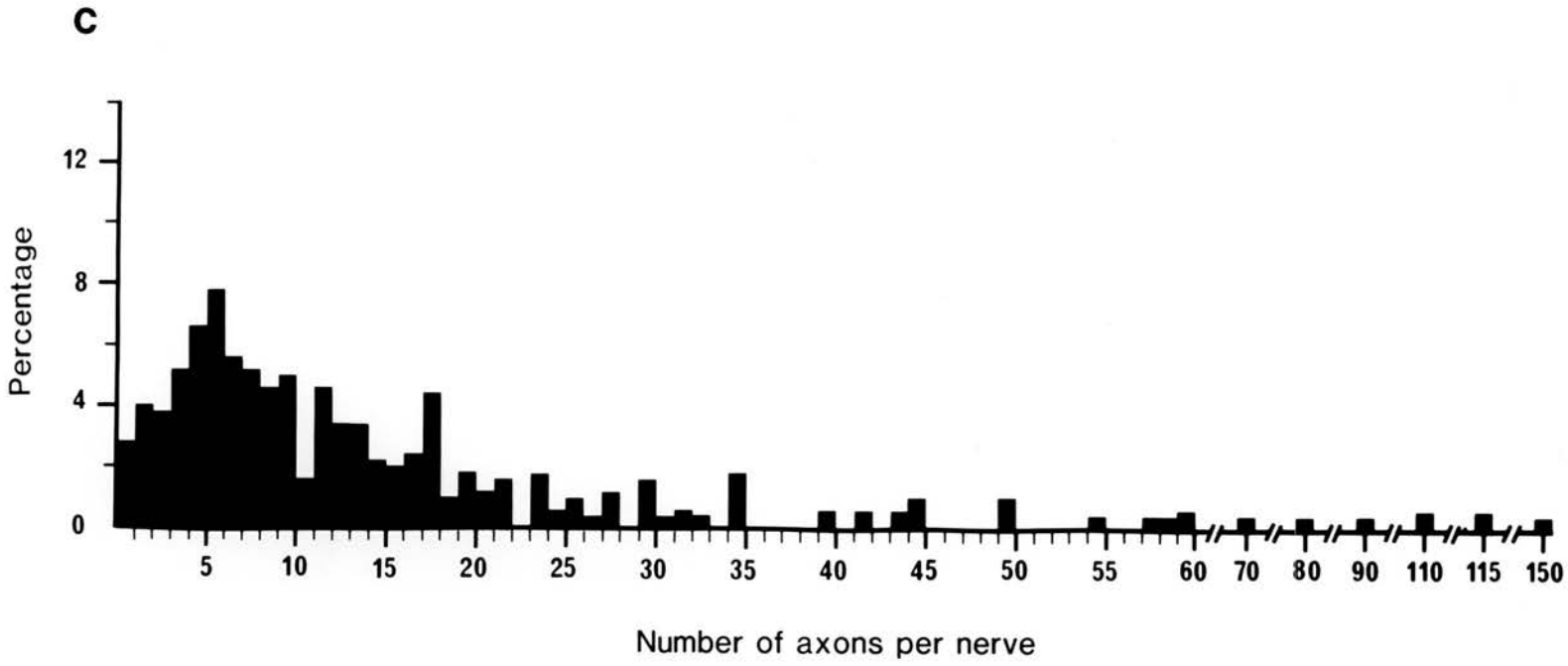
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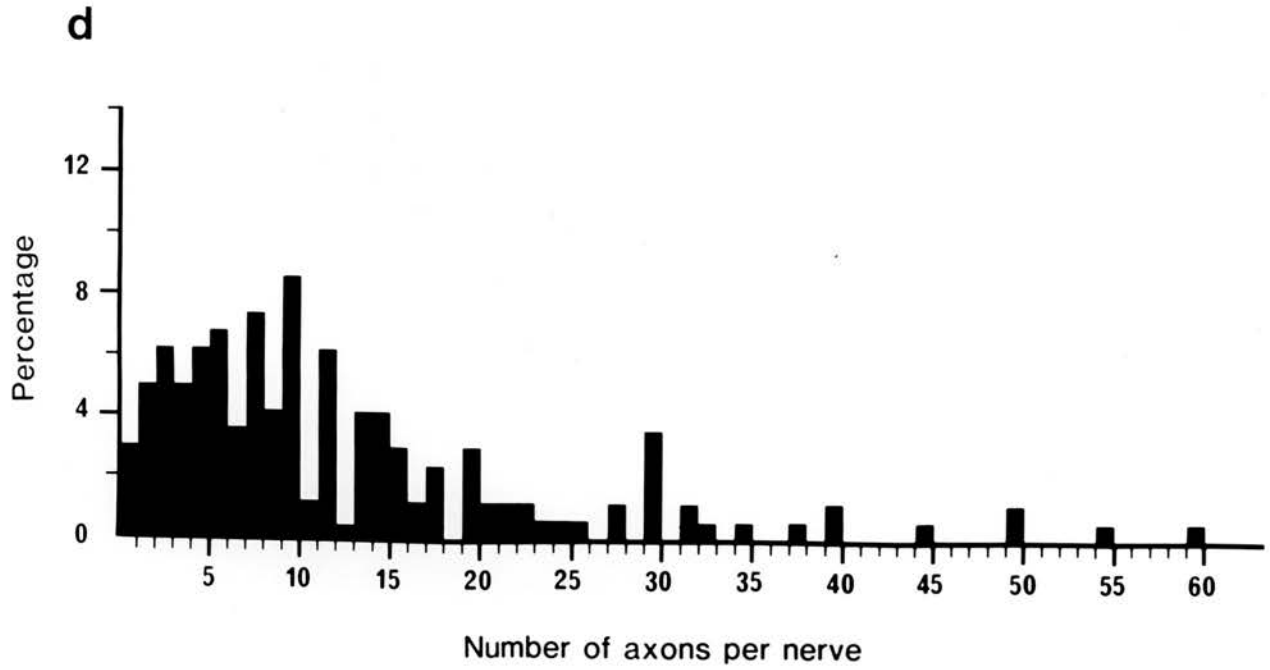
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cells and fibroblasts. Their structure and distribution were the same as that described on page 62 for the ileo-caeco-rectal junction.

(6) Quantitative Observations on the Innervation in the Region of the Recto-Coprodeal Junction.

The quantitative study (Table 15) showed that the innervation of the circular muscle at the recto-coprodeal junction (36 nerve bundles/ 1112 muscle fibres) was denser than the innervation of the circular muscle in the rectum 5 mm from the junction (23 nerve bundles/ 1080 muscle fibres), in the coprodeum 5 mm from the junction (20 nerve bundles/ 990 muscle fibres) and in the middle of the rectum (14 nerve bundles/ 1100 muscle fibres).

The total number of axon profiles counted per number of circular muscle cell profiles was 635 axons/ 1112 muscle cells at the recto-coprodeal junction, 399 axons/ 1080 muscle cells in the rectum 5 mm from the junction, 267 axons/ 990 muscle cells in the coprodeum 5 mm from the junction, and 207 axons/ 1100 muscle cells in the middle of the rectum.

The number of vesiculated axon profiles and their percentage of the total number of axon profiles was greater at the recto-coprodeal junction (163, 25.7%) than 5mm from the junction in the rectum (55, 16.2%) and coprodeum (47, 17.6%), and in the middle of the rectum (32, 15.5%).

Table 15

Distribution of nerve bundles and axon profiles in the circular muscle of the intestine in the region of the recto-coprodeal junction of ten birds.

Region	No. of circular muscle fibres	No. of nerve bundles	Total no. of axon profiles	No. of non-vesiculated axon profiles	No. of vesiculated axon profiles
Middle portion of the rectum	1100	14	207 (100%)	175 (84.5%)	32 (15.5%)
Rectum 5 mm from junction	1080	23	339 (100%)	284 (83.8%)	55 (16.2%)
Recto-coprodeal junction	1112	36	635 (100%)	472 (74.3%)	163 (25.7%)
Coprodeum 5 mm from junction	990	20	267 (100%)	220 (82.4%)	47 (17.6%)

The innervation of the circular muscle at the recto-coprodeal junction was significantly denser and contained more vesiculated axon profiles ($p \leq 0.01$) than in the rectum and coprodeum 5 mm from the junction (Table 16).

Table 16

Distribution of axon profiles and vesiculated axon profiles in the circular muscle of the intestine in the region of the recto-coprodeal junction of ten birds.

Region	Total no. of axon profiles	Statistical significance (t test)	No. of vesiculated axon profiles	Statistical significance (t test)
Middle portion of the rectum	207 (100%)		32 (15.5%)	
		t=3.38 (p<0.01)		t=5.22 (p<0.01)
Rectum 5 mm from junction	339 (100%)		55 (16.2%)	
		t=5.79 (p<0.01)		t=11.6 (p<0.01)
Recto-coprodeal junction	635 (100%)		163 (25.7%)	
		t=5.47 (p<0.01)		t=10.6 (p<0.01)
Coprodeum 5 mm from junction	267 (100%)		47 (17.6)	

VI. DISCUSSION

A. Gross Observations.

The present gross observations on the junction regions in the large intestine of the duck generally agree with the findings in the chicken (Calhoun, 1954; Hodges, 1974, p. 81; Clarke, 1978). The terminal part of the duck ileum is described for the first time in the present study as having a funnel-shaped lumen which decreases gradually in diameter and reaches its narrowest part at the the ileo-rectal junction. Most of the previous studies on the ileo-caeco-rectal junction in birds (Hill, 1971; Sturkie, 1976) have stated that the junction is formed by mucosal folds. Such folds were not observed in the present investigation which like Hodges (1974, p. 81) and Clarke (1978) in the chicken found that the junction is formed by a protrusion of the terminal part of the ileum into the lumen of the rectum.

In the chicken the base of each caecum has a narrow lumen with mucosal folds (Browne, 1922; Clarke, 1978). In the present study the openings of the caeca in the duck were found to originate on either side of the ileal projection ventro-laterally and had no mucosal folds. The diameter of the orifices was only 1.4-1.8 mm which is less than the 3-5 mm observed by Pilz (1937) in the same species. These conflicting measurements may be due to the belief that the caecal orifices are formed between the ileal projection and the lateral part of the base of each caecum. In the present study the caeca were found to

join the rest of the intestine obliquely, so that the medial and lateral parts of the caeca lie obliquely at different levels and project for a short distance into the lumen of the rectum. The size of the lumen at the base of each caecum is measured between these two points. The diameter of the rectum was also found to be diferent from that measured in the duck by Pilz (1937). This may have been because the measurements were not made in the same position, which in this study was close to the ileo-rectal junction. A mucosal fold which was observed by Liebe (1914) and Komarek (1969) at the recto-coprodeal junction described by them as having the capability of closing the junction was not seen. These gross observations have focused the attention on the possibility that muscular structures may be present which could be responsible for controlling the movements of the ingesta up and down the intestine. The diameter of the lumen at the ileo-rectal junction, at the entrances of the caeca and at the recto-coprodeal junction is much less than in the rest of the intestine and this could help in controlling the movements of the ingesta. In addition the caudomedial direction of the caecal orifices could facilitate the passage of the intestinal contents into the caeca during its retrograde flow along the rectum.

B. Nerve Supply of the Large Intestine.

The present observations on the innervation of the duck large intestine including the ileo-caeco-rectal junction and recto-coprodeal junction in general resemble the findings of Hsieh (1951, p. 103-106) in the chicken. The large intestine in birds is innervated by the intestinal nerve and in the chicken the cell bodies of the postganglionic cells are aggregated on the nerve to form large swellings known as ganglia. These ganglia are scattered along the course of the nerve at nearly regular intervals particularly in the region of the large intestine (Hsieh, 1951, p. 103; Watanabe, 1972; Akester, 1979, p. 405). Many other small nerve cell bodies appear to be scattered among these large ganglia (Akester, 1979, p. 405). In the duck in the present study the nerve cell bodies were found to be randomly distributed along the course of the nerve and very few ganglia were observed macroscopically close to the ileo-caeco-rectal junction suggesting that in the duck the cell bodies of the postganglionic cells on the nerve are too small to form gross swellings. Many more fine branches arising from these ganglia were observed to supply the area of the ileo-caeco-rectal junction than the rest of the large intestine suggesting that the activity at the junction requires greater nervous control. Unlike in the chicken in which the intestinal nerve is doubled above the rectum (Hsieh, 1951, p. 105), in the duck the nerve was found to be a single trunk throughout its course.

Obviously a macroscopic study like this is of limited value as it investigated

the caudal part of the intestinal nerve which supplies the large intestine and omitted the sympathetic and parasympathetic contributions to the cranial end of the nerve. Additionally, most of the branches of the splanchnic nerves, as well as other branches from the nerve plexuses in the region which contributed to the intestinal nerve, cannot be identified grossly since they are either too fine or hidden by fat. It is hoped, however, that the data on the innervation of the large intestine of the duck presented in this thesis will at least prove helpful to future studies employing more sophisticated experimental approaches.

C. Histological Study of the Musculature of the Ileo-Caeco-Rectal Junction and Recto-Coprodeal Junction.

The arrangement of the muscle at the junctions of the large intestine has been poorly investigated in birds. Most of the histological studies have been concerned with the general structure of the intestinal tract (Browne, 1922; Calhoun, 1954; Hill, 1971, p. 18; Hodges, 1974, p. 81; McLelland, 1979 , p. 149; Gabella, 1985). The only serious study on the musculature of the intestinal tract junctions appears to be that by Clarke (1978) in the ileo-caeco-rectal junction in the domestic fowl. The reconstruction method used to build circular muscle in the present study appears to be very effective in showing the thickness of the muscle in three-dimensions and making it clear that the muscle at this region is about three times as thick as in the adjacent areas. The

longitudinal histological sections were also very useful in exploring the muscle arrangement especially at the ileo-caeco-rectal junction. It is essential that in these longitudinal sections the terminal part of the ileum is included along with the origin of the caeca. Thus, if longitudinal sections are prepared parallel to the long axis of the intestine each caecum will be cut separately from the ileum and as a result the connection of the muscle at the base of the caeca will be separated from the muscle in the terminal part of the ileum.

Whilst most previous accounts of the ileo-caeco-rectal junction in birds with well-developed caeca (Hill, 1971; Sturkie, 1976; McLelland, 1979) infer that the junction is formed by flaps or valves, this description is not correct for the domestic duck since there are no structures at the junction in this species which could perform a passive flap-like closure. The ileo-caeco-rectal junction consists of a papilla-like protrusion of the ileum into the rectum. In the present study the term " ileal papilla " has been used to describe this protrusion, a term which has recently been adopted by the Nomina Anatomica Veterinaria (1983) for a similar structure in the domestic mammals. The papilla consists mainly of the ileal circular muscle.

The present study has demonstrated for the first time that in the domestic duck the circular muscle at the junction forms three thick rings or sphincters which are continuous with one another. These are an ileal sphincter in the ileal papilla, and right and left caecal sphincters around the orifices of the caeca. It

is interesting that in the duck the muscular rings at the caecal orifices protrude for a short distance into the lumen of the gut and cause a reduction of the size of the lumen. A well-developed muscular ring in the terminal part of the ileum was described in the chicken by Clarke (1978) but he was not able to describe any muscular rings at the orifices of the caeca. Furthermore, since he prepared the longitudinal histological sections of the caeca without including the terminal part of the ileum he was not able to observe the relationhip of the rings to the muscular ring at the terminal part of the ileum. Although the account of Clarke is the only study on the musculature of the ileo-caeco-rectal junction in birds he concluded that the terms " valve " or " sphincter " should be avoided because of their functional implications. In the present study the term " sphincter " has been used in a purely structural sense to describe three muscular rings. To what extent these sphincters are physiologically active cannot be deduced on purely anatomical grounds and must await functional studies. Moreover, the continuity that has been shown to exist between the three sphincters makes speculation even more difficult since it shows that each sphincter may not be acting independently from the other sphincters and that a highly specialised functional system or closing apparatus may be working at this site.

Little attention has been given in the literature to the musculature of the recto-coprodeal junction in birds (Calhoun, 1954) and most of the studies are centred on the presence of mucosal folds at the junction (e.g. Liebe, 1914; Weyrauch and Roland, 1958; Komarek, 1969; King, 1981). In the present

investigation a slightly thickened region of muscle was found at the caudal end of the rectum close to the junction. This has been called the " recto-coprodeal sphincter". Since the function of the sphincter at the caudal end of the rectum appears to act on the junction between the rectum and coprodeum the term " caudal rectal sphincter " was not used although a similar term " the lower oesophageal sphincter " has been employed at another site in mammals. The ability of this thickened muscle to produce sphincteric action again cannot be deduced on pure anatomical observations and this sphincter must also await physiological study. Nevertheless, in birds complex movements of the intestinal contents have been observed to occur between the coprodeum and rectum which suggests the presence of an active zone at the junction.

A well-developed muscularis mucosae has been described at the gastrointestinal junction in mammals (Dornhorst et al., 1954; Botha, 1958 a; Mann and Shorter, 1964). It was found that this muscle occurred in folds of mucous membrane and could therefore take part in the closing mechanism of the junction (Botha, 1958 a). The present study in the duck has shown something similar in that the muscularis mucosae is thickened in the ileal papilla, around the orifices of the right and left caeca, and at the recto-coprodeal junction. However, it was not included in mucosal folds. Certainly, contraction of this muscle could contribute to the sphincteric action of the anatomical sphincters at the two junctions.

The longitudinal muscle layer has been shown to be thickened at the regions of the gastrointestinal junctions in mammals (Horton, 1928; Botha, 1958 a; Cai and Gabella, 1984; Vaithilingam et al., 1984). Whilst at the pyloric sphincter in man the longitudinal muscle on the gastric side of the junction fuses with the pyloric circular muscle (Horton, 1928), in the guinea-pig, in contrast, the longitudinal musculature of the stomach and duodenum are directly continuous (Cai and Gabella, 1984). In the present investigation the longitudinal muscle layer is thickened close to the base of the ileal papilla and at the origins of the caeca. This muscle does not extend into the ileal papilla but immediately proximal to the base of the papilla becomes continuous caudally with the longitudinal muscle of the rectum and laterally with the longitudinal muscle of the right and left caeca. The longitudinal muscle in the region of the pyloric sphincter in man is considered by Horton (1928) and DiDio and Anderson (1968) to be a " dilator " of the junction. In the present study in the duck it is not possible to seriously consider the presence of dilator muscles in the sphincters at the junctions or their roles until physiological studies have been carried out. It has been shown electrophysiologically that the propagation of slow waves across the pyloric junction in the guinea-pig occurs along the longitudinal muscle (Fujii, 1971). However, from the light and electron microscopic studies after excision and resuture of the pylorus in the cat Johnson (1981, p.107) has shown that the gastro-duodenal activity was restored after three weeks when the nerve plexus had regenerated. Thus it seems that the

transmission of electrical impulses across the junction is most likely by a nerve plexus rather than by muscle, which supports the conclusion of Atanassova (1969) that this pathway is neurological. In the chicken electrical stimulation of the intestinal nerve at high frequencies results in contraction of the longitudinal muscle at the base of the caeca and in the rectum (Tindall, 1976). In the present study the longitudinal muscle of the ileum and caeca was found to be continued caudally by the longitudinal muscle of the rectum, whilst the longitudinal muscle of the caudal part of the rectum was continued caudally by the longitudinal muscle of the coprodeum. This could provide, in addition to the pathway by the myenteric plexus, a pathway for the transmission of slow waves and waves of contraction across the area of the junction. It is hoped that these observations on the arrangement of the musculature in the regions of the sphincters will be helpful to future physiological studies and might allow new experimental approaches to solve the problem of how these sphincters work.

D. Ultrastructure of the Muscle Cell at the Ileo-Caeco-Rectal Junction and Recto-Coprodeal Junction.

(1) Muscle layers.

The muscle layers in the terminal part of the ileum of the duck are basically similar to those of the chicken described by Gabella (1985) and to the arrangement in mammals (Gabella, 1972). In the chicken the wall of the small intestine consists of four layers which are directly apposed to each other with no structure equivalent to the submucosa found in the gut of reptiles, amphibians and mammals (Gabella, 1985). In the duck the submucosa of the small intestine is present but poorly developed and the inner longitudinal layer is separated from the inner circular layer by a very thin layer of connective tissue which is absent at many points. Here the muscle cells of the two layers are in close contact with each other. The inner longitudinal layer consists of an inner longitudinal part and an outer circular part in both birds (Calhoun, 1954; Clarke, 1978) and mammals (Lane and Rhodin, 1964). In the present study this layer in the terminal part of the ileum and in the large intestine is formed only by longitudinal muscle fibres. As noted by Gabella (1985) in the chicken, in contrast to the small intestine the large intestine has a well-developed submucosa and muscularis mucosae including the sphincter regions. The inner longitudinal muscle layer in the duck is similar if not homologous to

the muscularis mucosae of the mammalian gut and could have the same function acting both to shorten the intestine and to decrease its diameter (Lane and Rhodin, 1964).

The circular muscle layer of the terminal part of the ileum consists of an inner thin portion and an outer thick portion. The presence of the inner circular portion (accessory circular muscle) has previously been described in the small intestine of both birds (Clara, 1926; Rosenberg, 1940; Gabella, 1985) and mammals (Li, 1940; Silva et al., 1971; Gabella, 1972, 1974; Wong, 1980; Rumessen and Thuneberg, 1982, Rumessen et al., 1982; Faussone-Pellegrini and Cortesini, 1983). The present study agrees with these workers in observing in the inner thin portion in the duck small muscle cell profiles, very few nerve bundles, no interstitial cells or fibroblasts, and very few blood vessels. Different staining affinities in the circular layer have already been observed with the electron microscope in the chicken by Gabella (1985). Unlike in the present study, however, Gabella found that the inner part was less electron-dense than the outer part. It is interesting to note that variations in the staining affinity, even within the same species, have been reported in the inner accessory component of the circular muscle of the small intestine of mammals (Gabella, 1972; Rumessen et al., 1982; Faussone-Pellegrini and Cortesini, 1983). The significance of this division of the circular muscle in birds and mammals is not known but it worth noting that in the duck the inner layer was not found to be part of the sphincter of the ileal papilla. Since this layer is situated immediately

underneath the submucosa it is suggested in mammals that it could be affected by pressure changes in the lumen before any changes occur in the main circular muscle layer (Gabella, 1974). The same suggestion could not be applied to the inner circular layer in the duck until physiologial studies are carried out to clarify whether this layer of muscle has a special function in detecting any luminal changes, and using these changes, possibly through afferent nerve fibres, to cause a subsequent contraction of the bulk of the circular muscle. Moreover, to consider the presence of this layer as a general structural feature of the small intestine of birds needs further studies in other species. This is especially so since in the comparative study on several avian species by Clara (1926) it is unclear whether or not this layer was found in the gut of all the species investigated. The inner circular muscle is found to be absent in the large intestine of birds including the caeca and rectum just as it is in the large intestine of mammals. It is not possible at the moment to decide whether or not this layer in birds is homologous to the accessory circular muscle of mammals in spite of some similarities.

The ultrastructure of the muscle cells in the present study resembles in many aspects the ultrastructure of visceral muscle cells in mammals (Gabella, 1979, 1981). Whilst there is general similarity of the ultrastructure of the muscle in the different layers and in the different regions, differences may exist depending on the thickness, depth and functional activity of these, and further histochemical and biochemical investigations are required. The visceral muscle

cells in mammals and birds are packed with three types of filaments : thin, intermediate, and thick. These myofilaments form the contractile apparatus of the muscle cells. In the present study, thin filaments which are generally considered to consist of actin and thick filaments which it has been suggested represent myosin filaments (Nonomura, 1968; Kelly and Rice, 1969; Cooke et al., 1970; Gabella, 1979) appear to be consistently present. The appearance of the filaments depends on the preservation procedure used (Kelly and Rice, 1969; Burnstock, 1970) and on the state of stretch or contraction of the muscle cells (Kelly and Rice, 1969; Lowy and Small, 1970). This probably explains why intermediate filaments in the present study were found only in some preparations. Intermediate filaments of smooth muscle cells do not contain actin or myosin and their main constituent is a 5500 dalton protein (Cooke, 1976; Small and Sobieszek, 1977). Its amino acid composition is very similar to that of neurofilaments (Davison and Winslow, 1974). Comparable data are not available for birds and further study is required.

In mammals many authors have indicated that myofilaments enter the dense bodies and dense bands (Mark, 1956; Prosser et al.,1960; Lane, 1965) suggesting that these areas in the muscle cells may act as attachment sites for the myofilaments. The present study has shown the association of thin myofilaments with the dense bodies and bands which could have the same function as in mammals by providing attachment to a very large number of myofilaments.

(2) Cell junctions.

This appears to be the first investigation of muscle cell junctions in the sphincter regions of the intestinal tract of birds. Other observations on the muscle cells in the digestive tract of birds have been restricted to the gizzard and non-junctional regions of the ileum (Bennett and Cobb, 1969; Imaizumi and Hama, 1969; Gabella, 1985). In contrast, much information is available in mammals (Dewey and Barr, 1962, 1964; Revel et al., 1967; Tomita, 1967; Abe and Tomita, 1968; Barr et al., 1968; Uehara and Burnstock, 1970; Iwayama, 1971; Gabella, 1972, 1979; Gabella and Blundell, 1979).

As in mammals, the basic types of intercellular junction between smooth muscle cells in the intestine of the duck are nexuses and intermediate junctions. Nexuses are characterised by the close apposition of adjacent muscle cell membranes. The intercellular gap here is reduced substantially to about 2-3 nm and there is a three-layered appearance. The nexus may appear as either a tight junction in which the membranes appear to fuse or a gap junction. Possibly this difference in appearance may depend on fixation (Brightman and Reese, 1969; Cobb and Bennett, 1969; Uehara and Burnstock, 1970). Nexuses were found in the circular muscle layer of all the areas investigated in the present study and their percentage was approximately doubled in the circular muscle of the ileal sphincter, caecal sphincters, and recto-coprodeal sphincter. Since nexuses are thought to be the low-resistance areas for electrical conduction which is

known to occur between smooth muscle cells (Dewey and Barr, 1964, 1968; Abe and Tomita, 1968; Barr et al., 1968; Cobb and Bennett, 1969; Iwayama, 1971) this increase in their percentage is possibly due to the functional activity of the sphincter region which could require more contact points between muscle cells and more efficient electrical coupling. Moreover, at the sphincter regions some cells have more than one nexus and this again could be related to the high activity in these regions as well as to allow more intimate communication between two cells thus permitting free movement of ions and small molecules (Gilula et al., 1972; Fawcett, 1986, p. 270). It would also provide more contact between cells to spread excitation from one cellular unit to another throughout the muscle mass.

A remarkable difference in the distribution of nexuses has already been observed in the longitudinal muscle layer in mammals (Henderson et al., 1971; Daniel et al., 1976; Paton et al., 1976; Gabella and Blundell, 1979) and the chicken (Gabella, 1985). In the present study the few nexuses that were observed occurred only in the longitudinal muscle of the sphincter regions. The possibility that this uneven distribution was due to a technical artifact which could affect the longitudinal muscle and not the circular muscle cannot be completely ruled out. These conflicting observations suggest that there is a real variation in the distribution of the nexuses although it may be premature to compare the different smooth muscle in different layers from this point of view because of possible variations in function. It is well known in mammals that

the longitudinal muscle cells constitute functional bundles and are in some way electrically coupled (Kuriyama et al., 1967). Thus the idea that muscle cells lack nexuses will generally be acceptable only when a new mechanism for the morphological correlate of electrical coupling in these muscles is found (Gabella, 1972; 1981). The absence of nexuses seen in some regions of the intestine from the longitudinal muscle layer in the present study may indicate as in mammals that electrical coupling could be achieved by an unknown mechanism which is not involving gap junctions.

The muscle cells of the circular layer in the regions of the junctions of the large intestine in the duck come in contact with the muscle cells of the longitudinal layer. The same junctions have been described in chickens (Gabella, 1985) and mammals (Gabella, 1972). There is electrical evidence (Bortoff, 1965; Kobayashi et al., 1966; Connor et al., 1977; Taylor et al., 1977) that electrical coupling occurs between the circular and longitudinal muscles. This indicates that current conduction between the muscle layers exhibits cable-like properties and therefore the presence of these junctions could form low-resistance electrical connection areas and could explain the function of the circular muscle in conduction of slow waves in a longitudinal direction.

The intermediate junction is another type of intercellular connection observed between muscle cells in mammals (Gabella, 1972, 1981) and chickens (Gabella, 1985). In the present study intermediate junctions were observed in

all the muscle layers but they are numerous in the circular muscle layer, particularly in the sphincter regions where many such junctions were found between two adjacent muscle cells. In mammalian visceral muscle two forms of intermediate junction have been described (Gabella, 1981). Some are small and have an intercellular gap of about 20 nm. Others are large with a gap of about 60 nm which contains a layer of dense material. In the present study the two forms of intermediate junction were seen in the duck. Whether these forms represent separate types remains to be established. Since they occur between the dense bands of muscle cells where the thin myofilaments are inserted they are most likely to provide mechanical connection between muscle cells and do not appear to be capable of serving as points of transmission of electrical activity from cell to cell.

E. Ultrastructure of the Nerve Bundles at the Ileo-Caeco-Rectal Junction and Recto-Coprodeal Junction.

The present observations on the distribution of the nerve bundles in the intestinal tract show that the nerves are distributed mainly in the circular muscle layer, the longitudinal muscle being sparsely innervated. This agrees with other observations on the circular muscle of the rat duodenum (Lane and Rhodin, 1964; Wong, 1977), guinea-pig ileum (Gabella, 1972), fowl intestine (Gabella, 1985), toad intestine (Rogers and Burnstock, 1966) and oesophagus (Wong,

1973), fish intestine (Yamamoto, 1966) and stomach (Tan and Wong, 1980). In birds the use of cholinesterase and fluorescence histochemical techniques has revealed that nerve bundles are mainly restricted to the circular muscle layer particularly in the rectum where the circular muscle is best developed (Ali and McLelland, 1978). The distribution of cholinesterase-positive and fluorescent nerve fibres in the circular muscle of the intestine of birds appear to be basically similar to that in other vertebrates (Gabella and Costa, 1967; Read and Burnstock, 1969; Costa and Gabella, 1971).

A nerve plexus, the " plexus muscularis profundus " has been described in the circular muscle layer of the small intestine of mammals below the innermost portion (Cajal, 1911; Li, 1937, 1940; Silva et al., 1971; Gabella, 1972; Wong, 1977; Rumessen et al., 1982). The present study has demonstrated for the first time in birds that numerous medium to large-sized nerve bundles containing many vesiculated axon profiles and interstitial cells occur between the inner and outer portions of the circular muscle layer of the terminal part of the ileum. These nerve bundles correspond to what has been described in mammals as the " plexus muscularis profundus " and the same name could be applied to these nerve bundles in this position in birds. The inner portion of the circular muscle layer was found to be sparsely innervated and in many areas there were no nerve bundles at all. The poor innervation of the inner circular layer has also been reported in mammals (Gabella, 1972) and may be due to the fact that the layer is very thin and the cells generally lie close to the axon profiles in the adjacent

plexus muscularis profundus. Thus the neurotransmitters released from the axons of this plexus would be sufficient to effect a neuromuscular response.

The relatively poor innervation of the longitudinal muscle layer observed in birds would appear to be a characteristic feature of the intestinal innervation of vertebrates (Jacobwitz, 1965; Gabella and Costa, 1967; Read and Burnstock, 1969; Silva et al ., 1971). The longitudinal muscle layer in the duck, however, is well innervated at the regions of the ileal sphincter, the caecal sphincters and the recto-coprodeal sphincter. This is similar to findings in the taenia coli of the guinea-pig (Hollands and Vanov, 1965; Åberg and Eränkö, 1967; Gabella and Costa, 1967; Read and Burnstock, 1969), the longitudinal muscle of the rectum in the guinea-pig, cat and dog (Furness and Costa, 1973; Howard and Garrett, 1973), and the longitudinal muscle of the rat duodenum (Wong, 1975) and toad oesophagus (Wong, 1973). The longitudinal muscle layer of the rectum in the domestic fowl is also well innervated (Ali and McLelland, 1978). Read and Burnstock (1969) reported that the rich innervation of the taenia coli of the guinea-pig is necessary because of the relative thickness of the muscle here. The lack of innervation of the longitudinal muscle in other regions suggests that the transmitter acts on the muscle by diffusing from the myenteric plexus. This could be true in the duck for the poorly innervated regions of the longitudinal muscle. Moreover, the myenteric plexus in the duck, and as noted also by Gabella (1985) in the chicken, is located partly within the longitudinal muscle layer and partly between this layer and the circular layer. The muscle cells of

the longitudinal layer come close to the axon profiles of this plexus which could partly compensate for the lack of neuromuscular transmission by the reduced numbers of nerve bundles in the layer.

The present study on the ultrastructure of the nerve bundles appears to be the first investigation on the innervation of sphincter regions in the intestinal tract of birds. Other observations on the innervation of the muscle coat in birds (Bennett and Cobb,1969 a, b; Gabella, 1985) have been restricted to the gizzard and non-junctional regions of the small intestine. In contrast, a considerable amount of information is now available with the electron microscope about the innervation in other classes of vertebrates including mammals (Richardson, 1958, 1960; Taxi, 1961, 1965; Lane and Rhodin, 1964; Scofield, 1968; Baumgarten et al., 1970; Burnstock, 1970; Campbell, 1970; Cai and Gabella, 1984; Vaithilingam et al., 1984), amphibians (Thaemert, 1963; Royd et al., 1964; Rogers and Burnstock, 1966 b; Wong, 1973) and fish (Yamamoto, 1966; Tan and Wong, 1980).

The circular muscle of the intestinal tract in the duck is innervated by nerve bundles of unmyelinated axons. However, both myelinated and unmyelinated axons were reported to be present in the chicken gizzard (Bennett and Cobb, 1969 b) and fish stomach (Wong and Tan, 1978). The presence or absence of myelinated axons in the intestines varies considerably between the different animals classes since in mammals only unmyelinated axons have been reported

(Gabella, 1972). On the basis of evidence with the light microscope by Tan and Teh (1974) and the electron microscope by Wong and Tan (1978), it was concluded myelinated axons do not extend distal to the stomach. The presence of myelinated axons in the chicken and fish digestive tract and their absence in the mammals would suggest that they may be a characteristic feature of lower vertebrates. Further studies are necessary to establish this.

Single axon profiles are reported to be common in the region of the gastro-oesophageal junction of the monkey (Vaithilingam et al., 1984) and in the stomach of coral fish (Tan and Wong, 1980), although Gabella (1972) observed them rarely in the guinea-pig ileum. In the toad Rogers and Burnstock (1966 b) found single axons to be more frequent in the circular muscle layer of the large intestine than in the small intestine. The present study on the duck has shown that in this species there are differences in the density of single axon profiles in the circular muscle in the different junctional regions which were investigated. The greatest density occurs in the caecal sphincters (6.5%) and the least in the recto-coprodeal sphincter (2.8%). Whilst single axons may be in contact with only one muscle cell they can also contact 2-4 muscle cells. The functional implications of this are that a single axon would stimulate several muscle cells whilst one to one muscle-nerve contact could provide a more precise type of stimulation.

It has been found that if the neuromuscular contact is to be effective func-

tionally, the gap between the vesiculated axon profiles and the muscle cells must not exceed 300 nm (Bennett and Rogers, 1967). In the present study a few close junctions between 20-40 nm were observed at the base of the ileal papilla but they are less common than in the circular muscle of the intestine of mammals (Thaemert, 1963; Nagasawa and Mito, 1967; Vaithilingam et al., 1984), amphibians (Thaemert, 1963; Royd et al., 1964; Rogers and Burnstock, 1966) and fish (Tan and Wong, 1980). The great majority of vesiculated axon profiles in the circular muscle of the duck are more than 100 nm from a smooth muscle cell which is similar to the findings of Gabella (1972) in the circular muscle of the guinea-pig ileum. The gap between the vesiculated axons and smooth muscle could vary during muscle contraction and relaxation.

Many axon profiles have a terminal varicosity which is clearly distinguished by their large size and their vesicular content. The significance of the varicose structure of axons is unknown, apart from providing a greater number of terminals within the same length of axon. Varicose fibres run parallel to the smooth muscle cells, each axon contacting several muscle cells. A single muscle cell could be in contact with more than one axon profile which may be from the same nerve fibre or from different nerve fibres. The axon bundles divide until single axons are left. These axons lose their Schwann cell investment and sometimes come to be very close to the smooth muscle cells. This would explain in the present study why a high percentage of single axon profiles was observed in the sphincter regions.

According to the size of vesicle, three types of terminal profile were observed. The first type of profile contains predominantly agranular vesicles and has been frequently described in both birds and mammals (Taxi, 1958, 1965; Baumgarten et al., 1970; Gabella, 1972; Cook and Burnstock, 1976 a; Vaithilingam et al., 1984). Axon profiles which are known to be cholinergic, such as those at the motor end-plate, preganglionic axons in the sympathetic ganglia, and axons supplying the adrenal medulla, contain mainly small clear vesicles (Furness and Costa, 1980). It thus seems likely that similar axons in the duck intestine are cholinergic. However, some serotoninergic axons in the brain contain small agranular vesicles (Chan-Palay, 1975; Palay and Chan-Palay, 1975) and some axon terminals with agranular vesicles in the gut (Rothman et al., 1976; Jonakait et al., 1979) utilise 5- hydroxytrypotamine. It is therefore possible that not all of the axons containing small agranular vesicles in the intestine are cholinergic. Furthermore, the agranular vesicles described in the present and previous studies as electron-lucent may contain granular material of a certain chemical composition and the heavy metal of the fixatives or stains used may not have been able to preserve or stain them. It is not possible that these vesicles have released their contents at the moment of fixation otherwise they would have collapsed and been found close to the axon limiting membrane.

The second type of axon profile which has been observed contains many small granular vesicles and a varying number of agranular vesicles. These axons were described as adrenergic terminals (De Robertis and De Iraldi, 1961;

Grillo and Palay, 1962; Baumgarten et al., 1970; Gabella, 1972; Cook and Burnstock, 1976 a). Although in the present study many axon terminals with small granular vesicles have been observed, the ultrastructural identification of small granular vesicles within axon profiles is complicated by the difficulty of preserving the core of these vesicles (Gabella, 1972; Wong, 1977). This difficulty may be overcame by using a specific marker e.g 5 or 6 hydroxydopamine which increases the granularity of the vesicle (Wong, 1977; Vaithilingam et al., 1984). The presence of these adrenergic axon terminals in the ileal sphincter, caecal sphincters and recto-coprodeal sphincter in the duck is similar to the presence of the intramuscular adrenergic fibres in some of the sphincters of the alimentary tract in mammals e.g. the cardiac sphincter and the anal sphincter of the guinea-pig (Costa and Gabella, 1968, 1971; Furness and Costa, 1973), and the pyloric sphincter of the rat (Gillespie and Maxwell, 1971).

The third type of varicosity was observed to contain many large granular vesicles and many agranular vesicles. Similar axon profiles have been reported in the gut of mammals (Baumgarten et al., 1970; Gabella, 1972; Cook and Burnstock, 1976 a). According to Baumgarten et al. (1970) these axon terminals are peptidergic. They suggested that peptides might be stored in the large vesicles in these axons before it was actually demonstrated that peptides do indeed occur in the intestinal nerves (Furness and Costa, 1980). These axons they termed P-type. This suggestion that polypeptides might be stored in large vesicles has recently been proved since VIP has been isolated by immuno-

cytochemical studies in large vesicles (Larsson, 1977; Johansson and Lundberg, 1981). Axon profiles containing large numbers of mitochondria and thought to be sensory nerve endings (Burnstock and Iwayma, 1971; King et al., 1974) were not observed in the large intestine of the duck.

The non-neuronal cells observed in the present study were Schwann cells, interstitial cells and fibroblasts and the structure of these cells was basically similar to that described in other vertebrates by Baumgarten et al. (1970), Gabella (1972) and Cook and Burnstock (1976 b). There has in the past been considerable controversy about several aspects of these cells. However, it is now almost universally accepted that interstitial cells and fibroblasts as seen in the present study can be distinguished structurally from each other in many ways including the degree of development of the endoplasmic reticulum and the Golgi areas, and the presence or absence of granular vesicles. These structural differences may be an indication of functional differences. Despite the structural differences it is not clear whether interstitial cells and fibroblasts should be considered as separate cell types or different states of the same cell type and whether or not they have the same origin. Although small granule-containing cells and mast cells have also been described in the intestine of mammals (Cook and Burnstock, 1976 b) these cells were not observed in the present study.

Whilst many gap junctions have been observed between interstitial cells

and muscle cells in the stomach of chicken (Imaizumi and Hama, 1969) they were less frequently seen in the present study. The existence of gap junctions and the close contact of interstitial cells and fibroblasts with vesiculated axons may suggest that these cells could be involved in electrical coupling mechanisms by transmitting stimuli received from the axons to the smooth muscle cells (Rumessen et al., 1982). However, close contacts between interstitial cells and fibroblasts and their processes do not appear to have been described be fore. Furthermore, in the duck in the present study many interstitial cells rich in mitochondria were interposed between the vesiculated axons of the plexus muscularis profundus and muscle cells of both the outer and inner portions of the circular muscle layer of the terminal part of the ileum.

F. Quantitative Observations on the Muscle Cells and Nerve Bundles at the Ileo-Caeco-Rectal Junction and Recto-Coprodeal Junction.

(1) Muscle cells.

The present investigation has provided for the first time information on the size of the smooth muscle cells in the different muscle layers of the large intestine of birds and in particular in the regions of the intestinal junctions, information which is not available even for mammals. Muscle cell length has been measured by previous workers on cells isolated by maceration (Prosser

et al., 1960; Taxi, 1965; Cooke and Fay, 1972) or on serial transverse sections examined in the electron microscope (Yamauchi, 1964; Taxi, 1965; Bennett and Rogers, 1967; Merrillees, 1968; McGeachie, 1975; Gabella, 1976). It is obvious from the conflicting data obtained from these studies that cell length is an uncertain parameter of the muscle cells since the cells can shorten and elongate over a wide range. Thus it is difficult to compare the present findings with the measurements available for other classes of vertebrates.

The muscle cell length in the duck was found to vary in different parts of the intestine. Its range in the non-sphincter regions was similar to that in the guinea-pig taenia coli (Taxi, 1965) and mouse small intestine (Bennett and Rogers, 1967). At the base of the ileal papilla, around the caecal orifices and at the recto-coprodeal junction the length appeared to be greater than in the adjacent areas and the range here was similar to that in the guinea-pig described by Gabella (1976). The length decreased towards the distal part of the large intestine, the range here being generally lower than that in the intestine of mammals. The length of the cells opposite the recto-coprodeal junction was again apparently greater than in adjacent sites.

In mammals the volume of the muscle cells has been measured on serial electron micrographs(Merrillees, 1968) and by a stereological method (Gabella, 1976). In the present study using the method of Gabella (1976) the volume was evidently greater at the base of the ileal papilla, around the caecal orifices

and at the recto-coprodeal junction. In the non-sphincter areas the volume was generally similar to that in the small intestine of the chicken (Gabella, 1985) but it appeared to decrease towards the distal part of the large intestine and its range here became less than that in the intestine of birds and mammals.

It seems that the volume of the muscle cells is increased at the intestinal junctions where the muscle is thickened. The thickness of the muscle at these junction is possibly increased not only by there being more muscle cells but also possibly by the volume of the cells being greater. It appears from the present investigation that the size of the muscle cells is increased in areas where a high motor activity occurs since these junctions are concerned with contraction and relaxation of the muscle layers to control the passage of the intestinal contents.

(2) Nerve bundles.

Whilst information on the number of nerve bundles and axon profiles in the circular muscle of the gut is available for mammals (Lane and Rhodin, 1964; Gabella, 1972; Cai and Gabella, 1984; Vaithilingam et al., 1984), amphibians (Rogers and Burnstock, 1966) and fish (Tan and Wong, 1980), the present study appears the first to provide similar data for birds. The only quantitative studies which compare sphincter and non-sphincter regions are those by Cai and Gabella (1984) on the gastro-duodenal junction in the guinea-pig and Vaithilingam et al. (1984) on the gastro-oesophageal and gastro-duodenal

junctions in the monkey.

The axon bundles counted in these studies and the present investigation were obtained from single cross-sections of the circular muscle. However, it must be remembered that the majority of axons extend longitudinally along the muscle and therefore are likely to innervate many more muscle fibres.

The number of nerve bundles and axon profiles and the percentage of the vesiculated axon profiles counted in the circular muscle of the duck varied in the different regions of the large intestine. The circular muscle of the ileo-caeco-rectal junction and recto-coprodeal junction were the most richly innervated compared with regions 5 mm from the junctions. The density of innervation of the intestinal junctions in the duck more or less resembles that of the pyloric sphincter in the guinea-pig (Cai and Gabella, 1984) but differs from the innervation of the gastro-oesophageal junction and gastro-duodenal junction in the monkey (Vaithilingam et al., 1984) which are relatively highly innervated. The reason for this difference in innervation may be due to the presence of a high percentage (34%) of single axon profiles in the study of Vaithilingam et al. which counted as nerve bundles.

The large-sized nerve bundles of 100 to 150 axons were found only in the circular muscle of the ileal papilla, at the caecal orifices and at the recto-coprodeal junction. It seems that the number of the nerve bundles and axon profiles increases in regions of the intestine where the circular muscle is thickened. The

increase in the density of innervation in the regions of the junctions is probably related to the high motor activity in these regions. The quantitative technique used in this study therefore would appear to be useful, in not only confirming the presence of anatomical sphincters, but also in identifying sphincters when the thickening of the circular muscle is minimal as at the recto-coprodeal junction. The usefulness of this method in identifying anatomical sphincters at junctions where the thickening of the muscle is not conspicuous requires to be established. It is hoped that this demonstration of a high innervation of the sphincter muscle will be of value in planning the electrophysiological studies on the nervous control of these regions.

G. Interpretation of the Observations in Relation to the Available Data on Sphincter Function and Intestinal Motility.

The interpretation of the present observations in relation to the available information on sphincter function and intestinal motility is extremely difficult since physiological information on intestinal function and the nervous pathways which are involved in controlling it, unlike in mammals, is very limited for birds. Moreover, the few studies on the motility of the intestine in birds have either been concerned with characterising intestinal movement or the effect of nerve stimulation and drugs on motility (Hill, 1983). Very little effort has been made to correlate the two lines of approach.

The identification of the nerves involved in the different gut reflexes is one of the present problems in the study of sphincter function and intestinal motility. The main approaches used to solve this problem have involved investigating the histology, histochemistry and ultrastructure of the different intestinal nerve plexuses. Although these studies can provide the necessary information on the structure and distribution of the nervous elements they are not able to relate particular types of nerve fibres to particular functions. The study of the electrical activity of the large intestine of the chicken by Tindall (1976) is one approach which it is hoped will give relatively reliable information on the function of the nerve fibres in controlling the activity of the muscle layers. Possibly the information in the present study on the density and distribution of the nerve bundles in the muscle will be helpful in planning the future electrophysiological studies.

In the present investigation the highest densities of innervation were observed in the junctional regions of the large intestine. The functional implications of this high density may be related to the high motor activity of these regions since it is possible that there is a direct correlation between the density of the innervation and the motor activity of the gut. The high density of the nerve bundles and vesiculated axons at the ileo-caeco-rectal junction may be correlated with the activity of the region in controlling the passage of the

ingesta from the ileum to the rectum, preventing its reflux from the rectum to the ileum, and allowing the ingesta to pass by retroperistalsis from the rectum into the caeca and back again into the rectum in the direction of the cloaca (Akester et al., 1967; Fenna and Boag, 1974 a). The high density of nerve fibres and vesiculated axons observed at the junction between the rectum and coprodeum may be correlated with the powerful retroperistaltic waves observed here by Yasukawa (1959), Akester et al. (1967), Nechay et al. (1968) and Fenna and Boag (1974 a). The information on the density and distribution of the nervous tissue in the junctional and non-junctional regions of the large intestine will hopefully in future studies be correlated precisely with the activity of each region. The available information in the literature on the motor activity of the ileo-caeco-rectal junction and recto-coprodeal junction is not enough to get closer to solve the problem of how these regions act. The present observations, therefore, demonstrate the urgent need for electro-physiological studies on the sphincters.

The physiological roles of some of the nerves associated with the gastrointestinal tract of mammals are known in broad terms. The sympathetic non-adrenergic nerves, which are of extrinsic origin, inhibit the propulsion of material along the digestive tract by acting on intrinsic cholinergic nerves involved in the peristaltic reflex and by constricting the sphincters. Cholinergic nerves are involved in the excitatory component of the peristaltic reflex and in the excitatory pelvic pathways to the large intestine. The enteric inhibitory nerves

participating in the intrinsic reflexes can be activated via extrinsic pathways. These nerves are involved in reflex relaxation of the gut muscle which facilitates the passage of the contents in a rectal direction (Costa et al., 1976; Furness and Costa, 1979, 1980). A non-adrenergic inhibitory innervation, as well as a non-cholinergic excitatory innervation, has also been described in the chicken intestinal tract (Everett, 1968; Bartlet and Hassan, 1971; Tekwaki et al., 1977). The adrenergic innervation of the circular muscle of the sphincter regions of the mammalian intestine can inhibit the muscle either directly or indirectly by inhibition of the synaptic transmission of the myenteric plexus by suppression of the cholinergic excitation (Norberg, 1967; Gabella and Costa, 1967; Gillespie and Maxwell, 1971). This may also occur in birds since, as in mammals, the circular muscle of the sphincter regions is innervated by adrenergic fibres.

VII. SUMMARY

(1) The aim of the thesis was to investigate the presence of anatomical sphincters at the ileo-caeco-rectal junction and recto-coprodeal junction of the large intestine of birds using the domestic duck (Anas platyrhynchos) as the subject.

(2) The methods used to study the anatomy of the digestive tract sphincters, the evidence for the existence of sphincters in the large intestine of birds, and the anatomical evidence for the existence of sphincters at the gastro-oesophageal, gastro-duodenal, and ileo-caecal junctions of mammals were outlined.

(3) The detailed objectives were as follows.

(a) To study the arrangement of the muscle at the ileo-caeco-rectal junction and recto-coprodeal junction using light and transmission electron microscopy and, in the case of the ileo-caeco-rectal junction, by the construction of 3-D models of the circular muscle and by scanning electron microscopy.

(b) To study the ultrastructure of the muscle cell at the junctions and to provide quantitative data on their size by estimating the length and volume.

(c) To study the ultrastructure of the nerve bundles at the junctions and to provide quantitative data on the number of nerve bundles and axon profiles in the circular muscle layer.

(4) The arrangement of the muscle at the ileo-caeco-rectal and recto-coprodeal junctions was investigated in adult birds by means of gross observations, light

microscopy and reconstruction models.

(a) The muscle layers in the wall of the terminal part of the ileum consisted of four closely apposed layers including the inner longitudinal layer, the inner circular layer, the outer circular layer and the outer longitudinal layer.

(b) The muscle in the wall of the caeca and rectum consisted of three layers including the muscularis mucosae (inner longitudinal muscle layer), the circular muscle layer and the outer longitudinal muscle layer. The inner portion of the circular muscle layer was found to be absent in the large intestine.

(c) The muscle layer in the ileal papilla was composed only of the muscularis mucosae and the circular layer, the outer longitudinal muscle layer being absent. There was no evidence for the existence of cells which were characteristic of the inner portion of the circular layer. The muscle layers around the caecal orifice consisted of the muscularis mucosae, the circular layer and the longitudinal layer.

(d) The muscularis mucosae in the terminal part of the ileum was thickened at the base of the ileal papilla and was continuous laterally with the muscularis mucosae of the caeca. At the base of each caecum it was thickened and became continuous medially with the muscularis mucosae of the papilla and laterally with the muscularis mucosae of the rectum. At the recto-coprodeal junction the muscularis mucosae was slightly thickened and became continuous distally with the muscularis mucosae of the coprodeum.

(e) The longitudinal muscle of the ileum did not extend into the ileal papilla. Immediately proximal to the base of the papilla it was thickened and became continuous caudally with the longitudinal muscle of the rectum. At the base of each caecum the longitudinal muscle was thickened and became continuous caudally with the longitudinal muscle of the rectum. The longitudinal muscle of the recto-coprodeal junction was not thickened. It was continuous caudally with the longitudinal muscle of the coprodeum.

(f) The circular muscle of the terminal part of the ileum was massively thickened at the base of the ileal papilla forming a thick muscular ring, the ileal sphincter. The circular muscle of the caeca was thickened forming muscular rings at the caecal orifices, the right and left caecal sphincters. These three muscular rings were continuous. The circular muscle layer in the caudal part of the rectum was slightly thickened 1-2 mm cranial to the recto-coprodeal junction forming an obliquely orientated sphincter.

(5) The ultrastructure of the muscle cells was investigated in adult birds, by means of transmission electron microscope.

(a) The ultrastructure of the muscle cells was basically similar to the structure of the mammalian visceral muscle cells. The main part of the cell was occupied by three different types of myofilaments. Dense bodies were scattered throughout the cytoplasm among the myofilaments and associated with the thin myofilaments. Dense bands were wide and attached to large areas of the cell

membrane. The plasma membrane of the cell was lined with numerous regular vesicles, the caveolae. Smooth endoplasmic reticulum was observed in the form of long tubules or as a lace-like network. Other cytoplasmic contents included free ribosomes, rough endoplasmic reticulum, microtubules and centrioles.

(b) Two types of intercellular junction were identified. The gap junctions (nexuses) were found mainly in the circular muscle layer of the junction regions whilst the intermediate junctions were formed between the dense bands of cytoplasm of adjacent cells.

(c) These findings were discussed in relation to the available ultrastructural information of the muscle cells in the chicken and other classes of vertebrates.

(6) The size of the muscle cells in three adult birds was calculated by estimating their length and volume using the stereological method of Gabella (1976).

(a) The longest muscle cells at the ileo-caeco-rectal junction occurred in the circular muscle layer at the base of the ileal papilla and around the caecal orifices. The muscle cells at the base of the ileal papilla were tended to belonger than those in the ileum and rectum 5 mm from the junction.The muscle cells around the caecal orifice were longer than those in the caecum and rectum 5 mm from the junction. At the recto-coprodeal junction the muscle cells in the circular layer were evidently longer than those in the rectum and coprodeum 5 mm from the junction.

(b) The largest volume of the muscle cells occurred at the junctional re-

gions of the large intestine. The muscle cells at the base of the ileal papilla had an apparently larger volume than those in the ileum and rectum 5 mm from the ileo-caeco-rectal junction. The muscle cells around the caecal orifices had a generally larger volume than those in the caecum and rectum 5 mm from the junction. The muscle cells at the recto-coprodeal junction had a somewhat larger volume than those in the rectum and coprodeum 5 mm from the junction.

(c) The differences in the cell length and volume between the sphincter and non-sphincter regions were discussed and compared with similar data in other vertebrates.

(7) The distribution and the ultrastructure of the nerve bundles in the muscle layers was investigated in adult birds by means of the transmission electron microscope.

(a) Nerve bundles and vesiculated axon profiles were distributed mainly throughout the circular muscle layer and were rarely seen in the inner and outer longitudinal layers.

(b) Numerous nerve bundles, the plexus muscularis profundus, were observed in the connective tissue between the inner and outer portions of the circular muscle in the terminal part of the ileum.

(c) Small and large axon profiles were identified. Small axon profiles contained mainly microtubules and neurofilaments, whilst large profiles contained mainly granular and agranular vesicles. Three types of axon profile terminals

were described. The first type of profile contained predominantly agranular vesicles and was probably cholinergic. The second type of axon contained small granular vesicles and a varying number of agranular vesicles and was probably adrenergic. The third type of profile contained mainly large granular vesicles and many agranular vesicles and was probably peptidergic.

(d) The non-neuronal cells were Schwann cells, interstitial cells and fibroblasts. Schwann cells gave rise to many long, thin processes which ensheathed many axons. Some structural differences between the interstitial cells and fibroblasts were observed.

(e) These observations were discussed in the light of the available ultrastructural information in other classes of vertebrates.

(8) The density of innervation in the circular muscle of the junctional regions was estimated in ten adult birds by counting the number of nerve bundles and axon profiles, and the percentage of vesiculated axon profiles per approximately 1000 muscle cells.

(a) The muscular rings at the base of the ileal papilla and around the caecal orifices were more innervated than the non-thickened circular muscle of the ileum, caecum and rectum 5 mm from the ileo-caeco-rectal junction. The innervation of the circular muscle at the recto-coprodeal junction was denser than the innervation of the circular muscle in the rectum and coprodeum 5 mm from the junction.

(b) The total number of axon profiles was greater at the base of the ileal papilla and around the caecal orifices than in the circular muscle of the ileum, caecum and rectum 5 mm from the junction. At the recto-coprodeal junction the total number of axon profiles was also greater at the junction than in the rectum and coprodeum 5 mm from the junction.

(c) The number of vesiculated axon profiles and their percentage of the total number of axon profiles was significantly greater in the circular muscle at the base of the ileal papilla and around the caecal orifice than in the circular muscle of the ileum, caecum and rectum 5 mm from the junction. At the recto-coprodeal junction the number of the vesiculated axon profiles and their percentage was also significantly greater than in the rectum and coprodeum 5 mm from the junction.

(d) The innervation of the thickened rings at the base of the ileal papilla and around the caecal orifices was significantly denser and contained more vesiculated axon profiles than in the ileum, caecum and rectum 5 mm from the junction. At the recto-coprodeal junction the innervation of the circular muscle was also significantly denser and contained more vesiculated axons than in the circular muscle of the rectum and coprodeum 5 mm from the junction.

(9) The discussion of the present observations in relation to the available information on sphincter function and intestinal motility shows the need for physiological studies on the junctional regions of the avian large intestine.

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